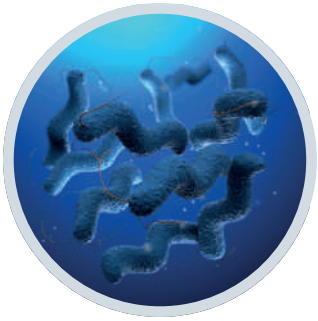
# MICROBIOLOGY MANUAL 2016/17



LAB™
A Neogen® Company

THE GATEWAY TO MICROBIOLOGY™

## ABOUT LAB M

Lab M specialises in microbiological culture media. The company develops, manufactures and supplies its products from its headquarters in Heywood, Greater Manchester, UK, and has an established network of distributors around the world. Lab M's customers include those in the food industry, water, environmental, clinical, pharmaceutical and vaccine manufacturers.

Lab M has an international reputation for the quality of its dehydrated culture media, which is available in various formats to support workflows and streamline efficiencies across different microbiology laboratories.

## OUR HERITAGE

Established in 1971, Lab M initially manufactured pre-poured media for clinical laboratories – and its name, London Analytical and Bacteriological Media, reflects its origins.

During the 1970s, the company developed its own dehydrated culture media, and by the early 1980s had added consumables and diagnostics to its products list. At that time Lab M was the only UK supplier offering such a range, and with a growing reputation for quality it soon became an international supplier.

Lab M has since evolved and relocated to a new purpose built head office and manufacturing facility in North West England, and now offer a comprehensive range of microbial products and solutions covering both traditional and rapid methods.

## PRODUCT QUALITY

The quality of Lab M's products has a direct impact on the service their customers provide. As a result of this Lab M consider quality to be everybody's responsibility.

Lab M is certified in accordance with ISO 9001:2008 & ISO 13485:2003 for the design, manufacture and supply of microbiological culture media, antibiotic supplements and diagnostic products. Lab M's products for the clinical market are supplied in compliance with the European IVD directive and carry the CE mark. Lab M's QC laboratory holds BS EN ISO 17025 accreditation by UKAS for the physical and microbiological performance testing of our ready-to-use Pinnacle™ media range.

These accreditations, alongside Lab M's stringent quality management system, ensure that only the highest standard of product is manufactured and distributed to our customers around the world.

## OUR BRANDS

HARLEQUIN™

Lab M´s Harlequin™ range of chromogenic media includes products for the isolation of Listeria species, Salmonellae, *Escherichia coli* and *Cronobacter sakazakii*.

µPREP™

Lab M's µPREP™ ready-to-reconstitute bagged dehydrated microbiological culture media are ready to use simply by adding water. This new range takes up minimal storage space, offers quick, convenient reconstitution with no need to autoclave.

PINNACLE™

The Pinnacle™ brand is a new line of ready-to-use plated culture medium, providing Lab M's high quality DCM prepared as ready-to-use plates under a stringent quality management system in a GMP environment.

CAPTIVATE™
Immunomagnetic Separation

Captivate™ antibody coated paramagnetic particles for the specific immunomagnetic separation (IMS) of micro-organisms. The range offers solutions for the concentration of a number of Shiga toxin-producing *Escherichia coli* (STEC) serotypes.

NutriTone™

NutriTone™ - Lab M offer a range of peptones for biotechnology applications. The NutriTone™ range is intended for use in mammalian cell culture. For a dedicated brochure please contact a member of the Lab M team.

OrganoTone™

OrganoTone™ is the brand name given to Lab M's range of media constituents. These include peptones for use in microbial fermentation by vaccine manufacturers, carbohydrates, selective agents and agars.

**FOR MORE INFORMATION** +44 (0) 161 820 3833 +44 (0) 161 820 5383

# *CONTENTS*

Lloyd's Register
LRQA

# CERTIFICATE OF APPROVAL

This is to certify that the Quality Management System of:

**Lab M Ltd**
**1 Quest Park, Moss Hall Road,**
**Heywood, Lancashire BL9 7JJ**
**United Kingdom**

has been approved by Lloyd's Register Quality Assurance
to the following Quality Management System Standards:

**ISO 9001:2008**

The Quality Management System is applicable to:

**Design, manufacture and supply of microbiological culture media, antibiotic supplements and diagnostic products. Supply of laboratory consumables.**

This certificate forms part of the approval identified by certificate number LRQ 0924069

Approval
Certificate No: LRQ 0924069/A

Original Approval: 3 June 1993

Current Certificate: 1 June 2014

Certificate Expiry: 31 May 2017

Issued by: Lloyd's Register Quality Assurance Limited

UKAS
MANAGEMENT
SYSTEMS
001

Hiramford, Middlemarch Office Village, Siskin Drive, Coventry, CV3 4FJ, United Kingdom.
This approval is carried out in accordance with the LRQA assessment and certification procedures and monitored by LRQA.
The use of the UKAS Accreditation Mark indicates Accreditation in respect of those activities covered by the Accreditation Certificate Number 001
Macro Revision 14

Lloyd's Register
LRQA

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Approval
Certificate No: LRQ 0924069/B

Original Approval: 3 June 1993

Current Certificate: 1 June 2014

Certificate Expiry: 31 May 2017

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Macro Revision 14

# Customised Media Service

**The Lab M Customised Media Service**

Since its inception, Lab M has been committed to customer needs by offering a wide range of quality media products for global markets. Our continued programme of product innovation and development has ensured that the Company has been responsive to market trends and changes in custom and practice. The increasing importance of regulatory compliance has also been a significant factor in this process of innovation.

From time to time, however, Lab M has been asked to 'design' media to meet specific customer applications. This has evolved into a more proactive approach to resolving customer needs:

**The Lab M Customised Media Service**.

**What do we offer?**

- Individually designed media, engineered to meet specific customer requirements.
- Close client collaboration.
- Development programmes that are both cost and time efficient.
- A process approach to development, commencing with feasibility tests, through product trials and into routine production.
- Customised packaging and labelling.
- Assurance of compliance with regulatory requirements.
- Total client confidentiality.
- Post-development consultancy.
- Commitment to on-going production.
- Quality products and service.

This is a unique service that differentiates us from our competitors and our Technical Support Group would be happy to discuss your needs on a confidential basis.

# Lab M Culture Media: The Process Outline

## RAW MATERIALS

**Agars, Peptones, Extracts, Dyes, Chemicals etc.**

**Each component is individually tested for suitability**

## PRODUCTION

**Weighing, Milling, Blending**

**Aproduction batch is made from raw materials of specified batch number which have been pre-tested for compatibility. The components are individually milled to ensure uniform particle size. Weighings are double checked before the components are blended.**

## QUALITY CONTROL

**Physical, Biological parameters. Comparison with previous batch and competition**

**Quality control first checks that the batch is completely blended, then a series of physical and biological tests are performed to ensure the product meets the exacting standards required by our customers. Comparisons with previous and competitor's batches are made. Results are recorded and a reference sample stored.**

## BOTTLING

**Into 500g sealed containers, or bulk containers at request**

**Automated equipment delivers pre-weighed amounts into containers which are hermetically sealed. Each container is immediately labelled with product details, code and batch number.**

## CUSTOMERS

**Lab M products are dispatched all over the world to microbiologists in all types of laboratory. Strict batch traceability in accordance with ISO9001 ensures we can recall all products if necessary, safeguarding your products/process.**

# Lab M Culture Media – The quality criteria

## Raw materials

**Peptones and Extracts –** Clarity, pH, moisture, growth promoting properties with Gram positive and Gram negative organisms aerobically and anaerobically, freedom from toxicity. Compatibility with other components, haemolysis patterns, antibiotic antagonists.

**Agar –** Clarity, pH, gel strength, melting point, setting point, heavy metal content (particularly $Ca^{++}$, $Mg^{++}$) compatibility with other components. Clarity on re-melt.

**Bile Salts –** Clarity, pH, thin layer chromatography, compatibility with other components.

**Dyes & Chemicals –** pH, chemical parameters, growth promotion inhibition, properties after incorporation into culture media.

## Production

All components from specified batch numbers. All components weighed accurately and checked.

Components milled to uniform particle size. Components blended for specified time, multiple samples taken to ensure thorough blending.

## Quality control

**Physical** – pH, clarity, gel strength, colour, heat stability, viscosity, redox.

**Biological** – Growth characteristics, productivity ratio, chemical reactions and colour changes, comparison with previous batch and competition.

# Preparing Culture Media

## Quality Assured

Before each batch of Lab M Culture media is passed for sale it undergoes a rigorous quality control procedure to ensure it gives maximum recovery and reproducibility. Reconstitution of media in the user's laboratory must be done with care to ensure the same high standards of performance.

The following section outlines the correct procedures which will ensure high quality reconstituted products, and suggests simple quality control techniques that can be used to check the performance of prepared media.

# Dehydrated Culture Media

## Storage

Dehydrated culture media stored unopened under optimal conditions have a shelf life of 2-5 years. In-house quality control by the user will help determine the condition of product in opened containers. The best conditions for storing dehydrated media are in a cool, even temperature away from any sources of moisture such as washing up areas or laboratory autoclaves and away from strong light. Storage in a refrigerator is generally not recommended (unless otherwise stated on the product label) as there is the risk of condensation on the container when it is brought out of the refrigerator. Storage instructions for Lab M media products are stated on the product label and must be followed.

**TABLE 1 – Deterioration of SS Agar stored in various conditions for 6 months.**

| Storage conditions | moisture gain % |
|---|---|
| Unopened bottle stored in cool, dark, dry conditions | 0 |
| Loose cap, stored in light on bench | 1.1 |
| Loose cap, stored in light in autoclave room | 4.4 |

The effect of the moisture gain on the performance of the agar can be quite dramatic. A 1.1% gain in moisture on storage will lead to a 53% reduction in the numbers of *Salmonella* isolated. Similarly a 4.4% gain in moisture will result in a 78% reduction in isolation rate. This demonstrates the importance of ensuring the container lid is tightly closed and the pot stored in cool, dry, dark conditions. *Barry, A. L. and Fay, G. D. A review of some common sources of error in the Preparation of Agar Media. (1972). Am. J. Med. Tech. Vol. 38 No. 7.* When a container is opened for the first time the date should be noted on the container. Dehydrated media should not be used if it shows any sign of moisture gain i.e. becomes lumpy or discoloured. The lid on the container should be replaced quickly after media has been taken out and closed tightly.

## Weighing Out

Using a top-pan balance with an accuracy of ±0.1 gram the powder should be spooned onto a weighing boat or clean beaker. Do not tip the media out of the container as this will cause excess dust which may be irritating and will certainly need cleaning up. The components of some formulations can be irritant so the wearing of a suitable face mask at this stage is advisable.

## Water

Purification by distillation, deionisation or reverse osmosis is advisable. It is important that the equipment is properly maintained; the output of ion resins need to be electronically monitored and microbial colonisation of the resin and tubing must be avoided. Storage vessels for purified water must also be monitored for microbial colonisation. It is advisable to use only fresh purified water with a conductivity of less than 10 microsiemens. Stored water tends to become acidic because it absorbs atmospheric $CO_2$. Tap water is not recommended because of the potential presence of heavy metal ions which can cause inhibition and precipitation problems.

# Quality Control of Culture Media

The routine quality control of culture media is an essential 'good laboratory practice' necessary to maintain the standards and performance of any bacteriological culture technique. Such practices are a key requirement for many laboratory accreditation schemes such as UKAS, and CLAS, INAB and ENAC etc. who accredit according to ISO17025

The International Standards Organisation recently published BS EN ISO 11133:2014, defining the requirements relating to the preparation, production, storage and performance testing of culture media that is intended for the microbiological analysis of food, feed and water. This applies to laboratories preparing media from dehydrated culture media (DCM) in-house, but also to media manufacturers. Lab M are compliant to this new standard, applying the appropriate QC criteria where applicable. This is reflected in the certificates of analysis provided by Lab M.

Lab M further supported this by gaining UKAS accreditation for their quality control laboratory according to ISO 17025:2005 in 2015. Lab M's schedule of accreditation covers both the physical and microbial performance testing of the Pinnacle™ media range; pH, sterility, fill volume, qualitative performance testing and quantitative performance testing. All methods are based on the new requirements of BS EN ISO 11133:2014.

## *Example of a typical quality control process*

A typical dehydrated culture medium (DCM) product will be subjected to a battery of tests. The manufacturer tests the product in its final prepared form (as a plate or broth) for all criteria. An end user needs only to perform a minimum QC assessment.

A typical manufacturer's testing regime is as follows:

**Determinants of physical quality.**

- Final pH.
- Clarity and presence of optical artefacts.
- Gel stability and consistency.

**Determinants of microbiological quality.**

Tests to assess microbial contamination and microbiological growth characteristics are required for each batch of end product.

**Microbial contamination.**

The samples tested include at least one plate or tube from the beginning, and one plate or tube from the end of a pouring or dispensing process. Plates or tubes are incubated for at least 18 hours under the routine incubation conditions specified for a particular media type. Target limits for the percentage of contaminated plates or containers of liquid medium should be established for each medium or specified by the manufacturer.

**Microbiological growth.**

Each batch of complete culture medium, nutrient components or supplements, are assessed for microbiological growth in terms of productivity, selectivity and specificity. Assessment may be by the quantitative, semi-quantitative or qualitative methods described in the standard, or by another generally accepted technique. Results are interpreted by comparing the amount of growth on the test medium with that on a specified reference medium. The growth of target strains should be typical in appearance, size and morphology, while the growth of non-target strains should be partly or completely inhibited.

**Test strains.**

Micro-organism cultures from the WDCM reference collection are documented in this standard, however it is stated that 'the use of equivalent strains from other culture collections is permitted'. Lab M typically test media with QC organisms drawn from ATCC, NCTC and NCIMB approved sources.

**Productivity.**

Solid, semi-solid or liquid culture media are inoculated with an appropriate inoculum of the working culture of each of the defined test micro-organisms. Productivity should reach a minimum limit as defined in the corresponding standard or as detailed in the technical specification. Quantitative methods require determination of the productivity ratio: a score of growth based on a comparison between the test medium and the defined reference medium.

**Selectivity and specificity.**

Quantitative assessment of selectivity requires inoculation of both selective culture media and a reference medium with the specified micro-organism, at an appropriate inoculum for testing. Selectivity, as defined by the selectivity factor, has to reach the value given in the corresponding standard or as set out in the technical specification. For semi-quantitative and qualitative methods, the growth of the non-target strain(s) should be inhibited partly or completely.

## *Assessment of growth*

### Solid Culture Media

**Productivity Ratio (P.R.)**

Productivity ratio is determined by assessing performance related to a control medium, which should be a nutritious agar such as Tryptone Soy Agar (LAB011). A controlled inoculum of approximately 100 colony forming units (cfu) must be used for both media and the P.R. is calculated by counting the colonies on the test and control media:

$$\text{P.R.} = \frac{\text{No. of colonies on test x dilution factor}}{\text{No. of colonies on control x dilution factor}}$$

Or to express as a percentage:

$$\text{P.R. (\%)} = \frac{\text{No. of colonies on test x 100}}{\text{No. of colonies on control}}$$

There are many inoculation methods that may be used to determine the productivity ratio, including:

- Spiral plate
- Miles-Misra and Modified Miles-Misra technique
- Pour plate
- Surface inoculation e.g. serial dilution surface incoulation using 'L-shaped spreader'

**Relative Growth Index (Ecometric Technique)**

The ecometric technique of Mossel is simple and gives numerical readings that can form the basis of records suitable for trend analysis. Both absolute growth index (AGI) and relative growth index (RGI) can be obtained by this method.

This plating technique is less-frequently used, but still offers a robust method of determining productivity. The ecometric technique is based on streaking an inoculum to extinction. The results obtained can be compared with previous batches of the same medium or with batches of the same medium from different manufacturers. The results can also be compared with results obtained using the same organisms on non-selective media.

### Liquid Culture Media

Liquid media are challenged with 10-100cfu of the target organism. Recovery of the organism is assessed qualitatively (visual) or semi-quantitatively (by sub-culture).

1. Prepare an overnight culture of the test organism in Tryptone Soy Broth (USP/EP/JP) (LAB004).
2. Prepare a tenfold serial dilution (to $10^{-12}$) in Maximum Recovery Diluent (LAB103).
3. Add 1ml of each dilution to 9ml of test and control broths. Incubate at 37°C for 18 hours.
4. Examine the broths and note the highest dilution showing growth (turbidity of the broth).

This method can be used in conjunction with the Miles-Misra technique to demonstrate recovery of known levels of CFU's in broth media.

## *References:*

BS EN ISO 11133:2014 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media

Mossel D.A.A. *et al* (1983) Quality Assurance of Selective Culture

# *Preservation of Stock Cultures*

The following method has been used in our laboratory for several years for long-term storage of microorganisms. For most strains a freezer at -20°C will suffice, however it should be noted that storage temperature may affect growth characteristics and viability.

## *Preservation of Bacteria using Protect™ Microorganism Preservation System*

### Culture Preparation

1. Grow the organism on an appropriate non-selective solid medium using the appropriate incubation temperature and atmospheric conditions to achieve a heavy growth. Use several plates with organisms forming small colonies.
2. Remove growth from plate using a sterile cotton swab and emulsify in the Protect™ cryopreservation fluid, making a thick suspension.
3. Carefully cap the vial and invert it six times. Leave the vial to stand for at least 30 seconds. . The vial may be tapped to remove the bubbles from bead centres.
4. Withdraw as much liquid as possible using a sterile, fine-tip pipette.

Cap the vial, label and place in freezer.

### Culture Recovery

1. Remove Protect™ vial from the freezer or liquid nitrogen container. Use a cryoblock, which has been stored in a freezer for at least thirty minutes to extend the time available for working with frozen vial.
2. Open vial. Remove one bead using sterile needle and recover the culture by rubbing bead over suitable agar medium or placing bead directly into broth. Removed beads must not be returned to the vial.

Duplicate vials can be prepared from the same original culture. This will assist should the vial become contaminated or should the pedigree of the organism be questioned.

The Protect™ Microorganism Preservation System is available to purchase from Lab M. Full details are available on the Lab M website, **www.labm.com**.

Details of recommended QC strains of organisms are contained in the individual entries for media.

# Microbiology Methods

There are numerous sources of information regarding microbiology methods, just some of which are listed below:

**Bacteriological Analytical Manual.**

Food and Drug Administration. Available online at **www.fda.gov**
(FDA's Bacteriological Analytical Manual (BAM) presents the agency's preferred laboratory procedures for microbiological analyses of foods and cosmetics. AOAC International published previous editions of this manual in a loose-leaf notebook format, and, more recently, on CD-ROM. This online BAM is now available to the public. Some changes have been made to methods since the previous version. A listing of chapters updated since the last hard-copy version (Edition 8, Revision A /1998) can be found in **About the Bacteriological Analytical Manual**)

**British Standards Institute.**
389 Chiswick High Road, London W4 4AL. www.bsigroup.co.uk

**Compendium of Methods for the Microbiological Examination of Foods 4th edition.**
2002. Edited by Downes, F.P. and Ito, K. American Public Health Association.
ISBN-10: 087553175X / ISBN-13: 978-0875531755

**European Pharmacopeia 8th Edition**.
European Pharmacopoeia, 2014, 8th edition, European Directorate for the Quality of Medicine, Council of Europe, 226 Avenue de Colmar BP907, F-67029 Strasbourg Cedex 1, France

**Manual of Microbiological Methods for the Food and Drink Industry, 5th edition**
2007. Campden BRI, Station Road, Chipping Campden, Goucestershire, GL55 6LD. Guideline G43. ISBN 978-0-905942-93-3.

**The Microbiology of Drinking Water**
2002. Methods for the Examination of Water and Associated Materials. Environment Agency. Available online from www. http://www.environment-agency.gov.uk/

**Practical Food Microbiology, 3rd edition**
2002. Edited by Roberts, D. and Greenwood, M. Wiley-Blackwell. ISBN-10: 1405100753 / ISBN-13: 978-1405100755

# Format and Abbreviation Guide

## *Product Name*

***(Alternative name or commonly used abbreviation)***

## Product Code

**Description:**
A brief outline which may include any of the following information on the medium:

- **History**
- **Mechanisms**
- **Applications**
- **Recognition By Regulatory/Advisory Bodies**
- **Advantages**

**Typical Formula:** The product composition in grams per litre; minor adjustments to the published formula may be made to meet performance criteria.

**Method for reconstitution**
Distilled water can be substituted for deionised water. "Allow to soak times" are not critical. If agar media are to be dispensed prior to sterilising, first bring to the boil to dissolve the agar.

**Appearance:** – of the finished cooled medium.

**pH:** at 20˚C. For agars, pour a small quantity into a universal bottle, allow to set and plunge the probe into the medium.

**Minimum Q.C. organisms** – for use every time a new batch of prepared medium is reconstituted. This short form check should not be confused with a full Q.C. of the medium. Where an organism should show inhibition this could be complete or partial. Records should be kept of these results to help recognise changes in performance over a period of time.

**Storage of Prepared Media** – All prepared media should be stored in the dark. If a medium is to be used beyond the suggested shelf life, appropriate quality control should be performed to demonstrate that there has been no detectable fall off in performance.

**Growth characteristics**

Abbreviation key for colonial descriptions:

| | |
|---|---|
| **CV** = convex | **CR** = crenated |
| **F** = flat | **Rz** = rhizoid |
| **E** = entire | **G** = glossy |
| **P.P.** = pinpoint | **D** = dull |

( ) brackets are used to denote occasional variations.

**References**
A list of related publications and sources of information.

N.B. The typical formulae in this manual and on the product label are adhered to wherever possible. However it is occasionally necessary to make minor adjustments to meet performance criteria.

# Culture Media Selection Guide

### Anaerobes

LAB160 Brazier's CCEY Agar
HP012 Columbia Agar (USP/EP/JP)
LAB001 Columbia Agar Base
LAB215 Columbia II Agar Base
LAB220 DRCM (ISO) - Differential Reinforced Clostridial Medium
LAB090 Fastidious Anaerobe Agar (F.A.A.)
LAB071 Fastidious Anaerobe Broth (F.A.B.)
HP001 Fluid Thioglycollate Medium (USP/EP/JP)
LAB425 Fluid Thioglycollate Medium (Clear)
LAB222 Iron Sulphite Agar
LAB109 Perfringens Agar (O.P.S.P.)
LAB194 Perfringens Agar (TSC)
LAB023 Reinforced Clostridial Agar
HP011 Reinforced Medium for Clostridia (USP/EP/JP)
LAB064 Thioglycollate Medium (Brewer)

### Bacillus

LAB020 Dextrose Tryptone Agar
LAB193 PEMBA (Bacillus Cereus Medium)
LAB073 PREP Bacillus cereus Medium

### Biomolecular

HAL004 Harlequin™ LB Agar
LAB168 LB Agar
LAB174 LB Agar (Lennox)
LAB169 LB Broth
LAB173 LB Broth (Lennox)
LAB191 Luria Bertani (Hi-Salt) Broth
LAB182 NZCYM Broth
LAB181 NZY Broth (NZYM)
LAB183 Terrific Broth
LAB175 YPD Broth
LAB176 YPD with Agar
LAB180 2xYT Agar
LAB179 2xYT Broth

### Blood Agar Bases

LAB028 Blood Agar Base
LAB015 Blood Agar Base No. 2
LAB001 Columbia Agar Base
LAB215 Columbia II Agar Base
LAB525 Eugon Agar
LAB090 Fastidious Anaerobe Agar (F.A.A.)
PIN007 Pinnacle™ Blood Agar No. 2 (25ml fill) Prepared
PIN006 Pinnacle™ Columbia Agar Base (25ml fill) Prepared
LAB011 Tryptone Soy Agar

### Blood Culture Media

LAB049 Brain Heart Infusion Broth
LAB071 Fastidious Anaerobe Broth (F.A.B.)
LAB004 Tryptone Soy Broth (USP/EP/JP)
LAB205 Tryptone Soy Broth (without dextrose)

### Brewing

LAB201 Lysine Agar
LAB198 Raka-Ray No.3 Agar
LAB199 Raka-Ray No.3 (Increased gel strength)
LAB079 W.L. Nutrient Agar
LAB038 Wort Agar
LAB200 Yeast & Mould Agar

### Clostridia

LAB160 Brazier's CCEY Agar
HP012 Columbia Agar (USP/EP/JP)
LAB001 Columbia Agar Base
LAB215 Columbia II Agar Base
LAB220 DRCM (ISO) - Differential Reinforced Clostridial Medium
LAB222 Iron Sulphite Agar
LAB109 Perfringens Agar (O.P.S.P)
LAB194 Perfringens Agar (TSC)
HP011 Reinforced Medium for Clostridia (USP/EP/JP)
LAB023 Reinforced Clostridial Medium Agar

### Coliform/Enterobacteriaceae

LAB051 Brilliant Green Bile 2% Broth
LAB091 E. E. Broth (Enterobacteriacae Enrichment Broth)
LAB060 Endo Agar
LAB061 Eosin Methylene Blue Agar
HAL008 Harlequin™ E. coli/Coliform Medium
HAL009 Harlequin™ mLGA
HAL003 Harlequin™ Tryptone Bile Glucuronide Agar (TBGA)
LAB126 Lactose Broth
LAB196 Lauryl Tryptose Broth
LAB045 MacConkey Agar No. 3
LAB005 MacConkey Broth (Purple)
LAB077 ColourScreen™ MLSTB-MT (ISO) Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan
PIN002 Pinnacle™ TBGA (Tryptone Bile Glucuronide Agar, TBX) Prepared
LAB072 Tryptone Bile Agar
LAB031 Violet Red Bile Agar (VRBA)
LAB088 Violet Red Bile Glucose Agar (VRBGA)
LAB573 VRBA with MUG

### Dairy

LAB081 CSEB - Cronobacter sakazakii Enrichment Broth
LAB060 Endo Agar Base
HAL012 Harlequin™ CSIM (ISO)
HAL013 Harlequin™ CSA-DFI - Cronobacter sakazakii Agar DFI Formulation
HAL012 Harlequin™ CSIM (ISO)
HAL010 Harlequin™ Listeria Chromogenic Agar
LAB126 Lactose Broth
LAB196 Lauryl Tryptose Broth
LAB092 M17 Agar
LAB019 Milk Agar
LAB115 Milk Plate Count Agar
LAB077 ColourScreen™ MLSTB-MT (ISO) Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan
LAB093 MRS Agar
LAB094 MRS Broth
PIN003 Pinnacle™ CSIM (ISO) Prepared
LAB098 Potato Dextrose Agar
LAB087 Sugar Free Agar
LAB063 Tryptone Glucose Extract Agar
LAB031 Violet Red Bile Agar (VRBA)
LAB088 Violet Red Bile Glucose Agar (VRBGA)
LAB573 VRBA with MUG
LAB038 Wort Agar
LAB099 Wort Broth (Modified)
LAB018 Yeast Extract Agar
LAB119 Yeast Extract Dextrose Chloramphenicol Agar

### Diagnostic Medical Microbiology

LAB195 BCYE Legionella Isolation Medium
LAB121 Bromocresol Purple Lactose Agar
LAB006 CLED (Bevis)-double indicator
LAB041 CLED (Mackey & Sandys)-single indicator
LAB090 Fastidious Anaerobe Agar
LAB067 GC Agar Base
LAB195 GVPC Legionella Isolation Medium (BCYE basal agar)
LAB027 Hoyle's Medium (Modified)
LAB035 TYC Medium

*Refer to other sections for our full range of Medical Products.*

### Diluents/ Isotonic solutions

HP017 Buffered Sodium Chloride-Peptone Solution pH 7.0 (USP/EP/JP)
LAB103 Maximum Recovery Diluent
LAB100Z Ringer's Solution (1/4 Strength) - Tablets

### Enteric Pathogens

LAB167 Aeromonas Agar
LAB013 Bismuth Sulphite Agar
LAB034 Brilliant Green Agar
LAB046 Buffered Peptone Water - pre-enrichment broth
LAB204 Buffered Peptone Water (ISO)
LAB112 Campylobacter Agar (Blood Free - Improved)
LAB135 Campylobacter Enrinchment Broth
CAP003 Captivate™ O26
CAP009 Captivate™ O45
CAP010 Captivate™ O91
CAP005 Captivate™ O103
CAP007 Captivate™ O104
CAP004 Captivate™ O111
CAP008 Captivate™ O121
CAP006 Captivate™ O145
CAP001 Captivate™ O157
LAB161 Sorbitol MacConkey Agar (SMAC)
LAB003 DCLS
LAB029 Desoxycholate Citrate Agar (DCA)
LAB065 Desoxycholate Citrate Agar (Hynes)
LAB537 Diassalm
HAL008 Harlequin™ E. coli / Coliform Medium
HAL009 Harlequin™ mLGA
HAL001 Harlequin™ Salmonella ABC Medium
HAL006 Harlequin™ Sorbitol MacConkey Agar (SMAC-BCIG)
HAL003 Harlequin™ TBGA
LAB110 Hektoen Enteric Agar
LAB116 MLCB Agar
LAB150 MSRV
LAB030 MacConkey Agar (with salt)

LAB002 MacConkey Agar (without salt)
HP006 MacConkey Agar (USP/EP/JP)
LAB216 MacConkey Agar No.2
HP005 MacConkey Broth (USP/EP/JP)
LAB202 Mueller Kaufmann Tetrathionate Novobiocin Broth (MKTTn)
LAB165 O157 Broth (MTSB)
PIN004 Pinnacle™mLGA Prepared
PIN002 Pinnacle™ TBGA (TBX) Prepared
PIN005 Pinnacle™ Salmonella ABC Medium Prepared
LAB086 Rappapport Vassiliadis Medium
HP007 Rappapport Vassiliadis Medium (USP/EP/JP)
LAB209 Rhamnose MacConkey (VTEC O26) Agar
LAB052 S.S. Agar (Salmonella Shigella Agar)
LAB044 Selenite Broth
LAB055 Selenite Cystine Broth
LAB096 TCBS Cholera Medium
LAB097 Tetrathionate Broth Base
MPB001 µPREP™ BPW (ISO)
LAB032 XLD Agar
HP008 Xylose Lysine Deoxycholate Agar (USP/EP/JP)
LAB120 Yersinia CIN Agar
LAB221 XLT4 Agar – Xylose Lysine Tergitol 4 Agar

## *Enterococci / Streptococci*

LAB028 Blood Agar Base
LAB015 Blood Agar Base No. 2
LAB207 Bile Aesculin Agar
LAB001 Columbia Agar Base
LAB215 Columbia II Agar Base
LAB106 Kanamycin Aesculin Azide Agar
LAB107 Kanamycin Aesculin Azide Broth
LAB216 MacConkey Agar No.2
LAB092 M17 Agar
PIN007 Pinnacle™ Blood Agar No. 2 (25ml fill)
PIN006 Pinnacle™ Columbia Agar Base (25ml fill)
LAB166 Slanetz and Bartley (m Enterococcus Medium)
LAB035 TYC Medium
LAB075 Todd Hewitt Broth

## *Food Microbiology*

LAB167 Aeromonas Agar
LAB085 Baird-Parker Medium
LAB285 Baird Parker Medium Base (ISO)
LAB207 Bile Aesculin Agar
LAB034 Brilliant Green Agar (Modified)
LAB582 Buffered Listeria Enrichment Broth PLUS
LAB046 Buffered Peptone Water
LAB204 Buffered Peptone Water (ISO)
LAB112 Campylobacter (Blood Free - Improved)
LAB135 Campylobacter Enrichment Broth
CAP003 Captivate™ O26
CAP009 Captivate™ O45
CAP010 Captivate™ O91
CAP005 Captivate™ O103
CAP007 Captivate™ O104
CAP004 Captivate™ O111
CAP008 Captivate™ O121
CAP006 Captivate™ O145
CAP001 Captivate™ O157
LAB081 CSEB - Cronobacter sakazakii Enrichment Broth
LAB218 DG18 Agar - Dichloran (18%) Glycerol Agar
LAB537 Diassalm
LAB217 DRBC Agar - Dichloran Rose Bengal Chloramphenicol Agar
LAB220 DRCM (ISO) - Differential Reinforced Clostridial Medium
LAB171 EC Medium (*E. coli* Medium)
LAB164 Fraser Broth
LAB212 Fraser Broth PLUS
LAB211 Half Fraser Broth PLUS
HAL013 Harlequin™ CSA-DFI - Cronobacter sakazakii Agar DFI Formulation
HAL012 Harlequin™ CSIM (ISO)
HAL008 Harlequin™ E.coli/Coliform Medium
HAL010 Harlequin™ Listeria Chromogenic Agar
HAL001 Harlequin™ Salmonella ABC Medium
HAL006 Harlequin™ Sorbitol MacConkey Agar (SMAC-BCIG)
HAL003 Harlequin™ Tryptone Bile Glucuronide Agar (TBGA)
LAB106 Kanamycin Aesculin Azide Agar
LAB107 Kanamycin Aesculin Azide Broth
LAB222 Iron Sulphite Agar
LAB196 Lauryl Tryptose Broth
LAB138 Listeria Enrichment Broth
LAB139 Listeria Enrichment Broth (Buffered)
LAB589 LEE Broth - Listeria Express Enrichment Broth
LAB122 Listeria Isolation Medium (Oxford)
LAB206 Listeria Isolation Media
LAB172 Listeria Monocytogenes Blood Agar (LMBA)
LAB216 MacConkey Agar No.2
LAB077 ColourScreen™ MLSTB-MT (ISO) Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan
LAB219 Modified Giolitti and Cantoni Broth (ISO)
LAB093 MRS Agar
LAB223 MRS Agar (ISO)
LAB094 MRS Broth
LAB150 MSRV
LAB202 Mueller Kaufmann Tetrathionate Novobiocin Broth (MKTTn)
LAB116 MLCB Agar
LAB165 O157 Broth (MTSB)
LAB147 Orange Serum Agar
LAB148 Palcam Agar
LAB144 Palcam Broth
LAB193 PEMBA - Bacillus Cereus Medium
LAB194 Perfringens Agar (TSC)
LAB109 Perfringens Agar (O.P.S.P.)
PIN003 Pinnacle™ CSIM (ISO) Prepared
PIN001 Pinnacle™ LCA Listeria Chromogenic Agar (ISO) Prepared
PIN002 Pinnacle™ TBGA (TBX) Prepared
PIN005 Pinnacle™ Salmonella ABC Medium Prepared
LAB149 Plate Count Agar
LAB010 Plate Count Agar A.P.H.A.
LAB098 Potato Dextrose Agar
LAB073 PREP Agar
LAB108 Pseudomonas Agar
LAB086 Rappaport Vassiliadis Medium (Broth)
LAB023 Reinforced Clostridial Agar
LAB209 Rhamnose MacConkey (VTEC O26) Agar
LAB055 Selenite Cystine Broth
LAB161 Sorbitol MacConkey Agar
LAB087 Sugar Free Agar
LAB097 Tetrathionate Broth Base A.P.H.A.
MPB001 µPREP™ BPW (ISO)
MPB004 µPREP™ Half Fraser Broth ISO (+FAC)
LAB155 UVM Broth
LAB031 VRBA
LAB573 VRBA with MUG
LAB088 VRBGA
LAB079 W.L. Agar
LAB038 Wort Agar
LAB099 Wort Broth (Modified)
LAB032 XLD Agar
LAB221 XLT4 Agar – Xylose Lysine Tergitol 4 Agar

## *Harmonised Pharmacopoeia (USP/EP/JP)*

HP017 Buffered Sodium Chloride-Peptone Solution pH 7.0 (USP/ EP/JP)
HP016 Casein Soya Bean Digest Agar (USP/EP/JP)
HP002 Casein Soya Bean Digest Broth (USP/EP/JP)
HP010 Cetrimide Agar (USP/EP/JP)
HP012 Columbia Agar (USP/EP/JP)
HP003 Enterobacteria Enrichment Broth – Mossel (USP/EP/JP)
HP001 Fluid Thioglycollate Medium (USP/EP/JP)
HP006 MacConkey Agar (USP/EP/JP)
HP005 MacConkey Broth (USP/EP/JP)
HP009 Mannitol Salt Agar (USP/EP/JP)
HP015 Potato Dextrose Agar (USP/EP/JP)
HP007 Rappaport Vassiliadis Salmonella Enrichment Broth (USP/EP/JP)
HP011 Reinforced Medium for Clostridia (USP/EP/JP)
HP014 Sabouraud Dextrose Agar (USP/EP/JP)
HP013 Sabouraud Dextrose Broth (USP/EP/JP)
HP004 Violet Red Bile Glucose Agar (USP/EP/JP)
HP008 Xylose Lysine Deoxycholate Agar (USP/EP/JP)

## *Identification Media*

LAB059 Kligler Iron Agar
LAB126 Lactose Broth
LAB054 Lysine Iron Agar
LAB104 Peptone Water
LAB069 Simmons Citrate Agar
LAB053 Triple Sugar Iron Agar
LAB129 Tryptone Water
LAB130 Urea Agar
LAB131 Urea Broth

## *Lactic Acid Bacteria*

LAB092 M17 Agar
LAB093 MRS Agar - de Man, Rogosa and Sharpe Agar
LAB223 MRS Agar (ISO)
LAB094 MRS Broth
LAB147 Orange Serum Agar
LAB198 Raka-Ray No.3 Agar
LAB199 Raka-Ray No.3 (Increased gel strength)

## *Listeria*

LAB139 Buffered Listeria Broth
LAB582 Buffered Listeria Enrichment Broth PLUS
LAB212 Fraser Broth PLUS
LAB164 Fraser Broth Base
LAB211 Half Fraser Broth PLUS
HAL010 Harlequin™ Listeria Chromogenic Agar
LAB138 Listeria Enrichment Broth
LAB589 LEE Broth - Listeria Express Enrichment Broth
LAB122 Listeria Isolation Medium (Oxford)

LAB206 Listeria Isolation Media
LAB172 Listeria Monocytogenes Blood Agar (LMBA)
LAB148 PALCAM Agar Base
LAB144 PALCAM Broth
MPB004 µPREP™ Half Fraser Broth ISO (+FAC)
LAB155 UVM Broth Base
PIN001 Pinnacle™ LCA (Listeria Chromogenic Agar)

### *Neutralising*

LAB188 D/E Neutralising Agar
LAB187 D/E Neutralising Broth
LAB186 D/E Neutralising Broth Base
LAB185 Letheen Agar (AOAC)
LAB184 Letheen Broth (AOAC)
LAB189 Microbial Content Test Agar

### *Nutrient Media for general use*

LAB048 Brain Heart Infusion Agar
LAB049 Brain Heart Infusion Broth
LAB525 Eugon Agar
LAB526 Eugon Broth
LAB008 Nutrient Agar
LAB068 Nutrient Broth 'E'
LAB014 Nutrient Broth No. 2
LAB062 Tryptose Phosphate Broth
LAB018 Yeast Extract Agar

### *Sensitivity Testing*

LAB039 Mueller Hinton Agar
LAB114 Mueller Hinton Broth
LAB012 Sensitivity Test Agar
LAB170 Susceptibility Test (Iso) Agar

### *Staphylococci*

LAB085 Baird-Parker Medium
LAB285 Baird Parker Medium Base (ISO)
LAB095 DN'ase Test Agar
LAB007 Mannitol Salt Agar
HP009 Mannitol Salt Agar (USP/EP/JP)
LAB219 Modified Giolitti and Cantoni Broth (ISO)
LAB192 ORSIM - Oxacillin Resistant Staphylococci Isolation Medium

### *Streptococci / Enterococci*

LAB028 Blood Agar Base
LAB015 Blood Agar Base No. 2
LAB207 Bile Aesculin Agar
LAB001 Columbia Agar Base
LAB215 Columbia II Agar Base
LAB106 Kanamycin Aesculin Azide Agar
LAB107 Kanamycin Aesculin Azide Broth
LAB216 MacConkey Agar No.2
LAB092 M17 Agar
LAB166 Slanetz & Bartley Medium (Membrane Enterococcus Agar)
LAB035 TYC Medium
LAB075 Todd Hewitt Broth

### *Sterility Test Media*

HP002 Casein Soya Bean Digest Broth (USP/EP/JP)
HP001 Fluid Thioglycollate (USP/EP/JP)
LAB425 Fluid Thioglycollate Medium (Clear)
LAB159 Malt Extract Broth
LAB014 Nutrient Broth No. 2
HP013 Sabouraud Dextrose Broth (USP/EP/JP)
LAB011 Tryptone Soy Agar
LAB004 Tryptone Soy Broth
LAB205 Tryptone Soy Broth (without dextrose)

### *Total Viable Counts*

LAB019 Milk Agar
LAB115 Milk Plate Count Agar
LAB010 Plate Count Agar A.P.H.A.
LAB149 Plate Count Agar
LAB163 R2A Medium
LAB063 Tryptone Glucose Extract Agar A.P.H.A.
LAB011 Tryptone Soy Agar
LAB197 Water Plate Count Agar (ISO)
LAB018 Yeast Extract Agar

### *Water Testing*

LAB224 Alkaline Saline Peptone Water (ISO)
LAB085 Baird Parker Medium Base
LAB207 Bile Aesculin Agar
LAB013 Bismuth Sulphite Agar
LAB048 Brain Heart Infusion Agar
LAB034 Brilliant Green Agar (Modified)
LAB051 Brilliant Green Bile 2% Broth

LAB195 BCYE Legionella Isolation Medium
LAB046 Buffered Peptone Water
LAB001 Columbia Agar Base
LAB537 Diagnostic Semi Solid Salmonella Agar (Diassalm)
LAB220 DRCM (ISO) - Differential Reinforced Clostridial Medium
LAB171 EC Medium
LAB060 Endo Agar Base
LAB061 Eosin Methylene Blue Agar (Levine)
LAB195 GVPC Legionella Isolation Medium (BCYE basal agar)
HAL009 Harlequin™ mLGA
HAL006 Harlequin™ SMAC-BCIG
LAB110 Hektoen Enteric Medium
LAB106 Kanamycin Aesculin Azide Agar
LAB126 Lactose Broth
LAB196 Lauryl Tryptose Broth
LAB054 Lysine Iron Agar
LAB005 MacConkey Broth (Purple)
LAB002 MacConkey Agar (Without Salt)
LAB030 MacConkey Agar (With Salt)
LAB216 MacConkey Agar No.2
LAB045 MacConkey Agar No 3
LAB103 Maximal Recovery Diluent
LAB082 Membrane Lauryl Sulphate Broth
LAB080 Minerals Modified Glutamate Broth
LAB008 Nutrient Agar
LAB014 Nutrient Broth No. 2
LAB165 O157 Broth (MTSB)
LAB109 Perfringens Agar (O.P.S.P)
LAB194 Perfringens Agar (TSC)
LAB010 Plate Count Agar A.P.H.A
PIN009 Pinnacle™ BCYE Legionella Medium (ISO) Prepared
PIN008 Pinnacle™ GVPC Legionella Medium (ISO) Prepared
PIN004 Pinnacle™ mLGA Prepared
LAB108 Pseudomonas Agar Base
LAB163 R2A Medium
LAB203 R2A Broth
LAB086 Rappaport-Vassiliadis Medium (RVS)
LAB100Z Ringer's Solution (1/4 Strength) Tablets
LAB044 Selenite Broth
LAB166 Slanetz & Bartley Medium (Membrane Enterococcus Agar)
LAB161 Sorbitol MacConkey Aga
LAB096 T.C.B.S. Cholera Medium
LAB097 Tetrathionate Broth Base APHA
LAB053 Triple Sugar Iron Agar
LAB129 Tryptone Water
LAB131 Urea Broth Base
LAB197 Water Plate Count Agar (ISO)
LAB032 XLD Agar
LAB221 XLT4 Agar – Xylose Lysine Tergitol 4 Agar
LAB018 Yeast Extract Agar

### *Yeasts and Moulds*

LAB117 DTM Dermatophyte Test Medium
LAB218 DG18 Agar - Dichloran (18%) Glycerol Agar
LAB217 DRBC Agar - Dichloran Rose Bengal Chloramphenicol Agar
LAB201 Lysine Agar
LAB037 Malt Extract Agar
LAB159 Malt Extract Broth
LAB089 OGYE Agar
LAB098 Potato Dextrose Agar
HP015 Potato Dextrose Agar (USP/EP/JP)
LAB036 Rose Bengal Chloramphenicol Agar
LAB009 Sabouraud Dextrose Agar
HP014 Sabouraud Dextrose Agar (USP/EP/JP)
HP013 Sabouraud Dextrose Broth (USP/EP/JP)
LAB111 Sabouraud Maltose Agar
LAB079 W.L. Nutrient Agar
LAB038 Wort Agar
LAB099 Wort Broth (Modified)
LAB119 Yeast Extract Dextrose Chloramphenicol Agar
LAB200 Yeast & Mould Agar
LAB175 YPD Broth
LAB176 YPD Agar

# 1. Dehydrated Culture Media

## *Aeromonas Agar*

***Bile Salt Irgasan Brilliant Green Agar***

**LAB167**

**Description**

Aeromonas Agar is a highly selective medium for the isolation of *Aeromonas* spp. from food, clinical and environmental samples. Based on the selective agents brilliant green and irgasan, this medium will not inhibit those strains of *Aeromonas* spp. sensitive to ampicillin used in other media.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 5.0 |
| Meat Peptone | 5.0 |
| Xylose | 10.0 |
| Bile Salts No.3 | 8.5 |
| Sodium thiosulphate | 5.44 |
| Irgasan | 0.005 |
| Brilliant green | 0.005 |
| Neutral red | 0.025 |
| Agar | 11.5 |
| **Grams per litre** | **45.5** |

**Appearance:**

**Powder:** fine, free-flowing, homogeneous, buff

**Finished medium:** clear, purple gel

**pH:** 7.0 ± 0.2

**Method for reconstitution**

Weigh 45.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by bringing to the boil. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

**Inoculation:**

Faecal specimens: Inoculate surface of medium directly, spreading for single colonies.

Samples requiring enrichment: Inoculate alkaline peptone water and incubate at 37°C for 18-24 hr. Subculture onto Aeromonas Agar, surface spreading for single colonies.

**Incubation:** Incubate plates aerobically at 37°C for 18-24 hr. Examine for typical colonies and confirm as *Aeromonas* spp.

**Storage: Dehydrated culture media:** 10-25°C away from direct sunlight.

**Prepared media:** 7 days at 2-8°C in the dark (may be extended if moisture tight packaging used).

Minimum Q.C. organisms: *Aeromonas hydrophila* WDCM 00063
*E. coli* WDCM 00013 (inhibited)

**Confirmation**

Typical colonies (translucent, pale green colonies 0.5-3.0mm diameter) should be confirmed as presumptive Aeromonas spp. by performing an oxidase test and inoculating into Hugh & Leifsons O/F medium.

- *Aeromonas spp*. will give a positive oxidase reaction and demonstrate both oxidative and fermentative metabolism.
- *Pseudomonas spp*. will also be oxidase positive, but do not possess fermentative metabolism.

An alternative method is to inoculate triple sugar iron tubes.

- *Aeromonas* will typically produce an acid butt (yellow) and an alkaline or unchanged slant (red).
- *Pseudomonas spp*. will remain unchanged in both the butt and slant.

To fully identify colonies as *Aeromonas spp*. the above tests should be supported using a proprietary kit such as API 20NE or Microbact 24E (other products may be available).

**Interpretation**

| Organism | Size | Shape | Colour |
|---|---|---|---|
| *Aeromonas* spp.* | 0.5-3.0 | CV.E.G | Translucent pale green |
| *Pseudomonas* spp. | 0.5-1.0 | CV.E.G | Translucent pale green |
| *S.aureus* | No growth | | |
| *E. coli* | No growth | | |

* The selective nature of the medium may mean occasional strains do not grow, or grow poorly.

## *Alkaline Saline Peptone Water (ISO)*

***Alkaline Peptone Water, Alkaline Saline Water***

**LAB224**

**Description**

Alkaline Saline Peptone Water (ISO) is a medium for the enrichment of *Vibrio* spp. from food and water samples according to ISO 21872:2007.

Originally described by Shread, Donovan & Lee as an enrichment broth for *Aeromonas* spp. and identified by Cruickshank as an effective medium for the enrichment of *Vibrio* spp., Alkaline Saline Peptone Water uses elevated pH and salt levels to provide a favourable environment for the enrichment of *Vibrio* spp.

Peptone provides essential vitamins, minerals, amino acids & nitrogen for growth requirements. Sodium chloride provides essential electrolytes for maintenance of the osmotic balance.

| Typical Formula | g/litre |
|---|---|
| Peptone | 20.0 |
| Sodium chloride | 20.0 |
| **Grams per litre** | **40.0** |

**Appearance:**

**Powder:** fine, free-flowing, homogenous, buff

**Finished medium:** clear, very pale straw coloured liquid

**pH:** 8.6 ± 0.2

**Hazard classification:** NR – Not regulated

**Method for reconstitution**

Disperse 40 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

**Storage: Dehydrated culture media:** 10-25°C.

**Final medium:** 7 days 15-20°C

**Inoculation & Incubation – as per ISO 21872**

Primary enrichment

Add test sample to 9ml prepared medium.

For large quantities, it may be necessary to pre-heat the prepared medium to 37°C before inoculation with the test portion.

Incubate the initial suspension as follows:

ISO 21872-1

- 37°C for 6 hours ± 1 hour for deep-frozen products
- 41.5°C for 6 hours ± 1 hour for fresh, dried or salted products

ISO 21872-2

- 37°C for 6 hours ± 1 hour

**LAB224**

Secondary enrichment

Transfer 1ml of primary enrichment culture (after incubation) into 10ml prepared medium.

Incubate the initial suspension as follows:

ISO 21872-1

- 41.5°C for 18 hours ± 1 hour

ISO 21872-2

- 37°C for 18 hours ± 1 hour

**Sub-culture**

Using a loop, sub-culture both the primary enrichment and secondary enrichments onto LAB096 Cholera TCBS Medium

Minimum Q.C. organisms:
*V.cholerae* WDCM 00136
*V.parahaemolyticus* WDCM 00037
*E.coli* WDCM 00013

**References**


Cruickshank R. (1968) Medical Microbiology. 11th ed. Livingstone Ltd, London, UK.

ISO/TS 21872-1:2007 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio* cholerae.

ISO/TS 21872-2:2007 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 1: Detection of species other than *Vibrio parahaemolyticus* and *Vibrio* cholerae.

Shread, P., Donovan, T.J. and Lee, J.V. (1991). Soc. Gen. *Microbiol*. Q. **8**. 184.


# *Bacillus Cereus Medium*

***Phenol Red Egg Yolk Polymyxin Agar (P.R.E.P.)***
***Mannitol Egg Yolk Polymyxin Agar***

**LAB073**

**Description**

Introduced by Mossel and his co-workers in 1967 for the enumeration of *Bacillus cereus* in foods, this formula was shown to be the most effective for this purpose by Inal in 1972. Two reactions on this medium differentiate *B. cereus* from other members of the Bacillus group, these are mannitol fermentation and lecithinase production. Mannitol fermentation on this medium produces a yellow colour, *B. cereus* is mannitol negative and produces red colonies. The lecithinase production of *B. cereus* is indicated by a white precipitate around the colonies. Polymyxin is added to suppress coliforms but some *Proteus* spp and Gram positive cocci may grow through.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 1.0 |
| Balanced Peptone No. 1 | 10.0 |
| D-Mannitol | 10.0 |
| Sodium chloride | 10.0 |
| Phenol red | 0.025 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 46 grams of powder, disperse in 900ml of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and aseptically add 100ml of X073 egg yolk emulsion and 2 vials of X074 Polymyxin.

**Appearance:** Pink, opaque gel.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *B. cereus* WDCM 00001
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** – up to 7 days at 2-8˚C in the dark.
**Inoculation:** Surface, spreading or streaking for single colonies.
**Incubation:** 30˚C aerobically for 24-48 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *B. cereus* | 3.0-4.0 | F.CR.D. | Pink, white halo |
| *B. subtilis* | 2.0-3.0 | F.CR.D. | Yellow |
| *B. licheniformis* | 2.0 | F.Rz.D. | Yellow |
| *E. coli* | no growth | | |
| *S. aureus* | 1.0 | CV.E.G. | Yellow (white halo) |

**References**


Inal, T.: Vergleictiende Untersuchungen über die Selektivmedien zum qualitativen und quantitativen Nachweis von Vacillus cereus in Lebensmitteln.

Mitteilung I. : Fleischwritsch, 51: 1629-1632 (1971). IV. Mitteilung: Fleischwritsch, 52: 1160-1162 (1972).

Mossel, D.A.A., Koopman, M.J. and Jongerius, E. (1967). Enumeration of Bacillus cereus in foods. Appl. Microbiol. 15: 650-653. Thatcher, F.S., Clarke, D.S. (1978) Micro-organisms in foods. Volume 1 second edition. University of Toronto.

BS5763 Part 1L:1994. ISO7932:(1993) 3/100


# *Baird-Parker Medium Base*

**LAB085**

**Description**

Originally introduced in 1962, this medium was developed by Baird-Parker to overcome the problems of recovering damaged *Staphylococcus aureus* from foodstuffs.

Baird-Parker medium is highly selective by nature, due to the presence of potassium tellurite and lithium chloride. Tellurite inhibits most coliforms and is also reduced to telluride by *S. aureus*, giving the typical black colonies. Glycine and sodium pyruvate are both used as growth factors by staphylococci while the pyruvate also neutralises any toxic peroxides that may be formed.

Unlike some commercially available preparations, Lab M Baird-Parker Medium can be used with either Egg Yolk Tellurite (X085) or Rabbit Plasma Fibrinogen (X086).

When Baird-Parker medium is used with Egg Yolk Tellurite X085, presumptive *S. aureus* appear as black colonies demonstrating lecithinase activity (an opaque zone around the colony) and lipase activity (a zone of clearing encircling the opaque zone). Suspected *S. aureus* colonies should be confirmed with RPF for coagulase or latex agglutination test.

Rabbit plasma fibrinogen (RPF X086) is a more specific alternative to egg yolk tellurite and allows the direct detection of coagulase-positive *S. aureus*. Typical *S. aureus* appear as black colonies surrounded by a zone of precipitation (demonstrating coagulase activity). This is recognised as the gold standard method for the identification of *S.aureus*. RPF overcomes any issues with atypical colony forms and its use means further confirmatory tests are not necessary.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Beef Extract | 7.5 |
| Yeast Extract | 1.0 |
| Lithium chloride | 5.0 |
| Glycine | 12.0 |
| Sodium pyruvate | 10.0 |
| Agar No. 2 | 20.0 |

**Method for reconstitution**

**For Baird-Parker Medium LAB085 with Egg Yolk Tellurite X085**

Weigh 65.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121°C for 15 minutes. Cool to 47°C and add 5% (50mL) X085. Mix well before aseptically pouring into sterile Petri dishes. Dry the agar surface prior to use.

**For Baird-Parker Medium LAB085 with Rabbit Plasma Fibrinogen (RPF) Supplement X086**

Weigh 6.55 grams of powder and disperse in 90mL of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121°C for 15 minutes. Cool to 47°C and add 1 vial of reconstituted X086. Mix well before aseptically pouring into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Final medium: opaque cream/pale fawn gel (with X085)

translucent, pale straw gel (with X086)

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* WDCM 00034
*Staphylococcus saprophyticus* WDCM 00159
*Escherichia coli* WDCM 00013

**Storage of Prepared Medium:** Dehydrated culture media: 10-25°C

Poured plates: LAB085+X085 upto 3 days at 2-8°C in the dark; LAB085+X086 use on day of preparation.

**Inoculation:** Surface inoculation.

**Incubation:**
LAB085+X085: 37°C aerobically for 48 hours.
LAB085+X086: 37°C aerobically for 24-48 hours.

**Growth characteristics(with X085)**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. aureus* | 1.0-3.0 | CV.E.G. | Black | Narrow opaque margin surrounded by a zone of clearing |
| *S. saprophyticus* | 0.5-2.0 | CV.E.G. | Black | (poor growth) |
| Other Coagulase negative staphylococci | 0.5-1.0 | CV.E.G. | Black | (no growth) |
| *Proteus* spp. | 0.5-2.0 | F.Rz.G | Brown Black | (no growth) |
| *Bacillus* spp. | 0.5-1.0 | F.Rz.D. | Brown | (no growth) |
| *Entero-bacteriaceae* | no growth | | | |

**Growth characteristics(with X086)**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| Coagulase positive *S. aureus* | 1.0-3.0 | CV.E.G. . . | White Grey Black | Narrow opaque zone of coagulase activity |
| Coagulase negative staphylococci | 0.5-2.0 | CV.E.G. . | White Grey Black | (poor growth) |
| *Proteus* spp. | 0.5-2.0 | F.Rz.G | Brown Black | (no growth) |
| *Bacillus* spp. | 0.5-1.0 | F.Rz.D. | Brown | (no growth) |
| *Entero-bacteriaceae* | no growth | | | |

**References**


Baird-Parker, A.C. (1962). An improved diagnostic and selective medium for isolating coagulase positive staphylococci. J. Appl. Bact. 25(1): 12-19.

Baird-Parker, A.C. and Davenport, E. (1965). The effect of Recovery medium on the isolation of S. aureus after heat treatment and after storage of frozen or dried cells. J. Appl. Bact 28: 390-402.

Ten Broeke, R. (1976). The Staphylococcus medium of Baird-Parker in practical use. The occurrence of coagulase-positive, egg yolk nonclearing staphylococci. Antonie van Leeuwenhoek 33: 220-236.

Smith, B.A. and Baird-Parker, A.C. (1964). The use of sulphamethazine for inhibiting Proteus spp. on Baird-Parker's isolation medium for Staphylococcus aureus. J. Appl. Bact 27(1): 78-82.

Beckers N J. et al (1984). Canad. J. Microbiol. 30: 470-474.

Sawhney D. (1986) J. Appl Bact. 61:149-155.


# *Baird-Parker Medium Base (ISO)*

**LAB285**

**Description**

For the isolation of coagulase-positive staphylococci. Formulated to ISO 6888-1 and compliant to ISO 6888-2 and ISO 6888-3.

Originally introduced in 1962, this medium was developed by Baird-Parker to overcome the problems of recovering damaged *Staphylococcus aureus* from foodstuffs. This version of the medium is formulated according to ISO 6888-1:1999+A1:2003 and is in compliance with ISO 6888-2:2003+A1:2003 and ISO 6888-3:2003.

Baird-Parker medium is highly selective by nature, due to the presence of potassium tellurite and lithium chloride. Tellurite inhibits most coliforms and is also reduced to telluride by *S. aureus*, giving the typical black colonies. Glycine and sodium pyruvate are both used as growth factors by staphylococci while the pyruvate also neutralises any toxic peroxides that may be formed.

As with Lab M's traditional Baird-Parker Medium, LAB085, and unlike some commercially available preparations, the new ISO formulated Baird-Parker medium can be used with either Egg Yolk Tellurite (X085) or Rabbit Plasma Fibrinogen (X086).

When Baird-Parker medium is used with Egg Yolk Tellurite X085, presumptive *S. aureus* appear as black colonies demonstrating lecithinase activity (an opaque zone around the colony) and lipase activity (a zone of clearing encircling the opaque zone). Suspected *S. aureus* colonies should be confirmed with RPF for coagulase or latex agglutination test.

Rabbit plasma fibrinogen (RPF X086) is a more specific alternative to egg yolk tellurite and allows the direct detection of coagulase-positive *S. aureus*. Typical *S. aureus* appear as black colonies surrounded by a zone of precipitation (demonstrating coagulase activity). This is recognised as the gold standard method for the identification of *S.aureus*. RPF overcomes any issues with atypical colony forms and its use means further confirmatory tests are not necessary.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of casein | 10.0 |
| Yeast extract | 1.0 |
| Meat extract | 5.0 |
| Sodium pyruvate | 10.0 |
| L-Glycine | 12.0 |
| Lithium chloride | 5.0 |
| Agar | 20.5 |

**Method for reconstitution**

*For Baird-Parker Medium LAB285 with Egg Yolk Tellurite X085*

Weigh 63.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121°C for 15 minutes. Cool to 48°C and add 5% (50mL) X085. Mix well before aseptically pouring into sterile Petri dishes. Dry the agar surface prior to use. Sulphamezathine may be added at 0.05g/L to suppress the swarming of *Proteus* spp.

*For Baird-Parker Medium LAB285 with Rabbit Plasma Fibrinogen (RPF) Supplement X086*

Weigh 6.35 grams of powder and disperse in 90mL of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121°C for 15 minutes. Cool to 48°C and add 1 vial of reconstituted X086. Mix well before aseptically pouring into sterile Petri dishes. Dry the agar surface prior to use.

## LAB285

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Final medium: opaque cream yellow gel (with X085) clear, straw gel (with X086)

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* WDCM 00034
*Staphylococcus saprophyticus* WDCM 00159
*Escherichia coli* WDCM 00013

**Storage:**

Dehydrated culture media: 10-25°C

Poured plates: LAB285+X085 upto 3 days at 2-8°C in the dark; LAB285+X086 use on day of preparation.

**Inoculation:**

LAB285+X085: surface inoculation as per user's validated methods.
LAB285+X086: surface inoculation or pour plate as per user's validated methods.

**Incubation:**

LAB285+X085: 37°C aerobically for 48 hours.
LAB285+X086: 37°C aerobically for 24-48 hours.

**Interpretation:**

LAB285+X085: Presumptive *S. aureus* colonies appear as black colonies demonstrating lecithinase activity and lipase activity. All black colonies (suspected *S. aureus*) should be confirmed with a coagulase test (RPF) or a latex agglutination kit.

LAB285+X086: Typical *S. aureus* appear as black colonies surrounded by a zone of coagulase activity.

**References**


ISO 6888-1:1999+A1:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium (includes amendment A1:2003).

ISO 6888-2:1999+A1:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using rabbit plasma fibrinogen agar medium (includes amendment A1:2003).

ISO 6888-3:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 3: Detection & MPN technique for low numbers.

Baird-Parker, A.C. (1962). An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. *J. Appl.* Bact. 25(1):12-19.

Smith, B.A. and Baird-Parker, A.C. (1964). The use of sulphamezathine for inhibiting *Proteus* spp. on Baird-Parker's isolation medium for *Staphylococcus aureus*. J. Appl. Bact. 27(1):78-82


# *BCYE Legionella Isolation Medium*

## LAB195

**Description**

BCYE (Buffered Charcoal Yeast Extract) Legionella Isolation Medium (LAB195) is a base medium used for the isolation of Legionella from clinical and environmental samples. This medium is based on the charcoal yeast extract formulation of Feeley *et al*.[1&2] The performance of this medium is further enhanced by the additions of ACES (N-2-acetamido-2-aminoethane - sulphonic acid) buffer and α-ketoglutarate as defined by Edelstein[3]. This medium is also detailed in internationally recognized methodology[4] for the isolation of *Legionella* spp. from water.

Specimens or samples are often heavily contaminated with other bacteria and consequentially a range of selective supplements have been developed to aid isolation. Lab M provide the GVPC supplement (X195) which is most effective for the isolation of *L. pneumophila*. It is recommended that this supplement is used in conjunction with heat and acid sample treatments, to further reduce the growth of non-*Legionella* bacteria.

This product contains the ACES buffer and ferric pyrophosphate in the base medium. This negates the need for complex freeze dried supplements. A complementary growth supplement is provided (X196) which contains the L-cysteine and α-ketoglutarate. In addition, an α-ketoglutarate supplement (X197) is also available for the preparation of confirmatory media for suspected *Legionella* colonies.

**Principle of isolation**

Water samples are concentrated either by membrane filtration or centrifugation (turbid samples may also be centrifuged). To reduce the growth of unwanted bacteria, separate portions of the concentrated sample may be subjected to heat and acid treatments. Treated and untreated portions are then inoculated onto *Legionella* selective media.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 10.0 |
| Charcoal | 2.0 |
| Ferric Pyrophosphate | 0.25 |
| ACES Buffer | 10.0 |
| Potassium Carbonate | 2.28 |
| Agar | 14.0 |

**Supplements**

| BCYE Growth Supplement (X196) | |
|---|---|
| **Typical Formula** | |
| L-Cysteine | 400mg |
| α-ketoglutarate | 1000mg |

| Presumptive ID (X197) | |
|---|---|
| **Typical Formula** | |
| α-ketoglutarate | 1000mg |

Add one vial per 500mL of sterilised medium as appropriate.

**Method for reconstitution**

**Maintenance (BCYE)**

Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilize by autoclaving at 110ºC for 10 minutes. Cool to 47ºC and aseptically add 2 vials of reconstituted growth supplement X196. Mix well and pour into Petri dishes.

**Presumptive Identification (BCYE no L-Cysteine)**

Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilize by autoclaving at 110ºC for 10 minutes. Cool to 47ºC and aseptically add 2 vials of reconstituted growth supplement X197. Mix well and pour into Petri dishes.

**pH:** 6.9 ± 0.1

**Inoculation:**
Surface inoculation, streak for single colonies.

**Incubation:**

Incubate at 36 + 1ºC in a humid atmosphere under aerobic conditions for up to 10 days.

**Interpretation:**

Typical morphology should be regarded as presumptive *Legionella*.

Presumptive isolates should be confirmed using a serological method, e.g. Microgen M45 Latex.

Minimum Q.C. organisms:
*Legionella* spp. - Growth

**References**


Feeley, J.C., Gibson, R.J. *et al.* (1979). *Journal of Clinical Microbiology* **10**: 437-441

Pesculle, A.H., Feeley, J.C. *et al.* (1980). *Journal of Infectious Disease* **141**: 727-732

Edelstein, P.H. (1982). *Journal of Clinical Microbiology* **14**: 298-303

International Standard. ISO 11731:1998(E). Water Quality – Detection & Enumeration of *Legionella*.


## *Bile Aesculin Agar*

**LAB207**

**Description**

For the isolation and presumptive identification of Enterococci / Group D Streptococci. The aesculin produced by organisms positive for aesculin hydrolysis reacts with ferric citrate to form a dark brown or black complex. Bile salts inhibit Gram-positive organisms other than Enterococci or Group D Streptococci. This medium can also be used for presumptive differentiation of the Klebsiella-Enterobacter-Serratia group from other *Enterobacteriaceae*.

| Typical Formula | g/litre |
|---|---|
| Peptone | 8.0 |
| Bile salts | 20.0 |
| Ferric citrate | 0.5 |
| Aesculin | 1.0 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 44.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121ºC. Cool to 47°C, mix well and dispense into Petri dishes..

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff.

Finished medium: Buff/pale brown gel.

**pH:** 7.1 ± 0.2

**Hazard classification**
Xi – Irritant

Minimum Q.C. organisms:
*Enterococcus faecalis* WDCM 00087
*Enterobacter aerogenes* WDCM 00175
*Streptococcus pyogenes*

**Storage:**

Dehydrated culture media: 10-25ºC.

Poured plates: 7 days at 2-8°C in the dark.

**Inoculation:** Surface inoculation as per user's validated methods.

**Incubation:** Incubate at 37°C for 18-24 hours.

**Interpretation**

| organism | colony size (mm) | observations |
|---|---|---|
| *Enterococcus* spp. | 0.1 - 0.3 | Blackening of media around colony |
| *Enterobacter* spp. | 1 - 2 | Blackening of media around colony |
| *Pseudomonas aeruginosa* | 0.3 - 0.6 | |
| *Escherichia coli* | 1.5 - 2.5 | |
| *Stahpylococcus aureus* | 0.5 | |
| *Streptococcus pyogenes* | Inhibited | |

## *Bismuth Sulphite Agar*

***(Wilson and Blair Medium)***

**LAB013A + LAB013B**

**Description**

A modification of Wilson and Blair's original medium for the isolation of *Salmonella typhi* and other *Salmonella* from clinical samples, sewage and other materials. The presence of bismuth sulphite and brilliant green make this medium highly selective. As the medium contains neither lactose nor sucrose it can be used to detect lactose and sucrose fermenting *Salmonella.*

| Typical Formula | g/litre |
|---|---|
| **Bismuth Sulphite Agar Base 'A' LAB013A** | |
| Beef Extract | 6.0 |
| Balanced Peptone No. 1 | 10.0 |
| Ferric citrate BPC | 0.4 |
| Brilliant Green | 0.01 |
| Agar No. 2 | 20.0 |
| **Bismuth Chemical Mixture 'B' LAB13B** | |
| Bismuth ammonium citrate | 3.0 |
| Sodium sulphite | 5.0 |
| Disodium phosphate | 5.0 |
| Glucose | 5.0 |

**Method for reconstitution**

**Agar Base 'A':** Weigh 36.4 grams of powder and mix with 1 litre of deionised water. Sterilise for 15 minutes at 121˚C. Cool to 50˚C approx. and add 100ml of Chemical Mixture 'B'. Mix well and pour thin plates. Store at 4˚C for 3 days to mature, before use.

**Chemical Mixture 'B':** Suspend 18 grams of powder in 100ml of deionised water. Bring to boil over a tripod and gauze, and cool quickly in cold water. Add to 1 litre of Agar Base 'A' prepared as above.

**Appearance:** Pale green, opaque gel.

**pH:** 7.6 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – store 3 days before use. Use within 7 days. Store at 2-8˚C in the dark.

**Inoculation:** Surface, streak out to single colonies.

**Incubation:** 37˚C for 24 hours aerobically.

**Growth characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. typhi* | 1.5-2.0 | CV.E.G. | Black | Metallic sheen black deposit in medium. ($H_2S$-ve strains green) |
| Other *Salmonella* spp. | 1.0-2.5 | CV.E.G. | Black/ Green | Metallic sheen especially in heavy growth, single colonies may give rabbit eye appearance |
| *E. coli* | P.P.-1.0 | CV.E.G. | Green | |
| *Klebsiella* spp. | P.P-2.0 | CV.E.G. | Green | |
| *Citrobacter* spp. | 1.0-2.5 | CV.E.G. | Green | (black centre) |
| *Proteus* spp. | 1.0-2.5 | CV.E.G. | Green / Brown | (black centre) |

**References**


Wilson, W.J. and Blair, E.M. M'V (1926). A combination of bismuth and sodium sulphites affording an enrichment and selective medium for the typhoid-paratyphoid groups of bacteria. J. Pathol. Bacteriol., 29: 310-311.
International Journal of Food Microbiology (1987) 5:3:200-202.
I.C.M.S.F. (1978) Micro organisms in Foods I. Their significance and enumeration. 2nd edition Univ of Toronto Press. Speck M.L. Compendium of methods for microbiological examination of foods. (1984) 2nd edition. American Public Health Association, Washington. 3/102


## *Blood Agar Base*

**LAB028**

**Description**

An inexpensive general purpose agar base which, with the addition of 5% sterile blood, can be used to cultivate a wide range of micro organisms of clinical significance. Typical haemolysis patterns are obtained with this medium.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 10.0 |
| Balanced Peptone No. 1 | 10.0 |
| Sodium chloride | 5.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 37 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C and add 5-7% sterile defibrinated blood. Mix by swirling the flask and pour into Petri dishes.

**Appearance:** Dependent upon blood additive.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: S. *aureus.* WDCM 00034
*S. pyogenes* ATCC 19615

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking to single colonies.

**Incubation:** 37˚C aerobically, anaerobically or microaerobically for 24 hours.

**Growth characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. aureus* | 0.5-1.5 | CV.E.G. | White-Golden | haemolytic |
| *S. pyogenes* | P.P.-1.0 | CV.E.G. | Grey | beta haemolytic alpha haemolytic non-haemolytic |
| *S. pneumoniae* | P.P.-1.0 | F.E.G. | Grey | alpha haemolytic draughtsman |
| *N. meningitidis* | P.P.-1.5 | CV.E.G. | Grey | mucoid |
| *E. coli* | 1.5-2.5 | CV.E.G. | Grey | haemolytic |
| *Ps. aeruginosa* | 0.5-3.0 | F.CR.D. | Grey | many colonial forms green pigment |
| *B. fragilis* | 0.5-1.5 | CV.E.G. | Grey | mucoid |

**References**


Cruikshank, R. (1972). Medical Microbiology. 11th edn. Livingstone, London


## *Blood Agar Base No. 2*

**LAB015**

**Description**

A very rich agar base which, with the addition of blood, is capable of growing delicate clinical pathogens. The medium gives colonial appearances, haemolysis patterns and pigment production of diagnostic value. When the blood is 'chocolated' the medium gives good recovery of *Haemophilus* spp. The medium can be made selective for various groups by the addition of appropriate antibiotic mixtures eg:

Streptococci – Colistin/Oxolinic acid (X013)
*Gardnerella* spp. – Colistin/Oxolinic acid (X011)
*C. perfringens* – Neomycin (X015) (X016)
Staphylococci/streptococci – Colistin/Naladixic acid (X012)

| Typical Formula | g/litre |
|---|---|
| Tryptose | 15.0 |
| Soy Peptone | 2.5 |
| Yeast Extract | 5.0 |
| Sodium chloride | 5.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 39.5 grams of powder, disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 47˚C then aseptically add 5-7% sterile, defibrinated horse or sheep blood. Mix well before pouring.

**Appearance:** Dependent upon blood additive.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00034
*S. pyogenes* ATCC 19615

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking out to single colonies.

**Incubation:** 37˚C aerobically or microaerobically for 24 hours, anaerobically for 24 and 48 hours.

**Growth characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. aureus* | 1.5-2.0 | CV.E.G. | White/ Golden | (haemolytic) |
| *S. pyogenes* | 1.0-1.5 | CV.E.G. | Grey | beta haemolytic) (alpha or non haemolytic) |
| *S. pneumoniae* | 0.5-1.0 | F.E.G. | Grey | (draughtsman (alpha haemolytic) (mucoid) (require $CO_2$) |
| *N. meningitidis* | 0.5-1.0 | CV.E.G. | Grey | (May require $CO_2$) |
| *E. coli* | 2.0-3.0 | CV.E.G. | Grey | (haemolytic) |
| *Ps. aeruginosa* | 1.0-3.0 | F.CR.D. | Grey | (green pigment (haemolytic) |
| *B. fragilis* | 1.0-1.5 | CV.E.G. | Grey | non haemolytic |

## *Brain Heart Infusion Agar*

**LAB048**

### Description

A general purpose nutritious agar base. This medium was first used for the isolation of dental pathogens. With the addition of 7% defibrinated blood the medium will support the growth of a wide range of fastidious organisms, the phosphate buffer will help neutralise the acids produced from the utilisation of glucose and thus maintain viability. The medium is not recommended for the determination of haemolytic reactions because of the glucose content.

| Typical Formula | g/litre |
|---|---|
| Brain-Heart Infusion Mixture | 17.5 |
| Tryptose | 10.0 |
| Glucose | 2.0 |
| Sodium chloride | 5.0 |
| Disodium phosphate | 2.5 |
| Agar No. 2 | 12.0 |

### Method for reconstitution

Weigh 49 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes then swirl to mix. Sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C then pour into Petri dishes.

**Appearance:** Pale Straw colour, clear gel.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00034
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Surface, streaking out to single colonies.

**Incubation:** Time and temperature to suit specimen/organisms.

**Growth characteristics (with horse blood)**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *S. aureus* | 1.0-1.5 | CV.E.G. | White/ Golden |
| other Staphylococci | 0.5-1.5 | CV.E.G. | White/ Yellow |
| *S. pyogenes* | 0.5-1.0 | CV.E.G. | White |
| *E. faecalis* | 1.0-1.25 | CV.E.G. | Grey/ Green |
| *S. pneumoniae* | 0.5-1.0 | F.E.G. | Grey/ Green |
| *E. coli* | 2.0-3.0 | CV.E.G. | Grey |

### References


Roseburg. T., Epps, L.J. and Clarke, A.R. (1944). A study of the isolation, cultivation and pathogenicity of *Actinomyces israeli* recovered from the human mouth and from actinomycosis in man. J. inf. Dis., 74: 131-149.

Howell, E. (1948) Efficiency of methods of isolation of *Histoplasma capsulatum.* Pbl. Hlth. Rep. 63: 173-178. 3/108


## *Brain Heart Infusion Broth*

**LAB049**

### Description

A rich isotonic infusion medium with tryptose (a mixture of meat and milk peptones) providing a wide range of substrates. A low concentration of glucose is used to stimulate early growth. The medium is lightly buffered to prevent the early death of some species due to acid production. Organisms which produce significant amounts of acid may well overwhelm the buffering system and auto-sterilise. The medium is suitable for use as a blood culture medium or as an enrichment broth for fastidious organisms.

| Typical Formula | g/litre |
|---|---|
| Brain-Heart Infusion Mixture | 17.5 |
| Tryptose | 10.0 |
| Glucose | 2.0 |
| Sodium chloride | 5.0 |
| Disodium hydrogen phosphate | 2.5 |

### Method for reconstitution

Weigh 37 grams of powder then disperse in 1 litre of deionised water. Allow to stand for 10 minutes then dissolve with gentle heat before dispensing into tubes or bottles. Sterilise at 121˚C for 15 minutes. Overheating will cause caramelisation and darkening of the medium.

**Appearance:** Straw colour, clear liquid.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00034
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation:** (as a blood culture medium). Using a minimum volume of 50ml of medium add the blood to a dilution of from 1:10 to 1:20. Use in conjunction with an anaerobic culture medium e.g. Fastidious Anaerobe Broth LAB071.

**Incubation:** 37˚C aerobically for 7 to 15 days.

**Interpretation:** Observe daily, subculture after 1, 2, 3, 7 and 15 days or immediately on showing signs of growth.

### References


Rosenow. E.C. (1919). Studies on selective localisation; focal infection with special reference to oral sepsis. J. Dent. Res. 1:205-267.


## *Brazier's CCEY Agar*

**LAB160**

### Description

Brazier's CCEY agar is the formulation currently used by the Anaerobe Reference Unit for the isolation of *C.difficile,* resulting from work initiated by Ken Phillips and Paul Levett, and completed by Jon Brazier.

Based upon the market leading anaerobe medium, Fastidious Anaerobe Agar, it incorporates additional ingredients to improve the isolation and differentiation of *C.difficile* from clinical specimens.

Cholic acid is present to promote spore germination following alcohol shock treatment, and p-hydroxyphenylacetic acid to enhance the production of p-cresol, a distinctive metabolite of *C.difficile.*

Selectivity is achieved by addition of supplement X093 (cefoxitin cycloserine), whilst egg yolk emulsion X073 is added to help differentiate *C.difficile* from lecithinase positive clostridia. Finally, the addition of lysed horse blood optimises the recognition of colony fluorescence when cultures are examined using UV light.

| Typical Formula | g/litre |
|---|---|
| Peptone Mix | 23.0 |
| Sodium chloride | 5.0 |
| Soluble Starch | 1.0 |
| Agar No. 2 | 12.0 |
| Sodium bicarbonate | 0.4 |
| Glucose | 1.0 |
| Sodium pyruvate | 1.0 |
| Cysteine HCl | 0.5 |
| Haemin | 0.01 |
| Vitamin K | 0.001 |
| L-arginine | 1.0 |
| Soluble pyrophosphate | 0.25 |
| Sodium succinate | 0.5 |
| Cholic acid | 1.0 |
| p-Hydroxyphenylacetic acid | 1.0 |

**Method for reconstitution**

Weigh 48 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and aseptically add the following: 2 vials of X093, 40ml of Egg Yolk Emulsion X073 and 10ml lysed horse blood. Mix well and pour into Petri dishes.

**Appearance:** Tan opaque gel.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *C. difficile*
*E. coli* (inhibition) WDCM 00013

**Storage of prepared medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface streak untreated or alcohol shocked specimens for single colonies.

**Incubation:** 37˚C for 24-48hrs under anaerobic conditions

**Characteristics of *C.difficile:*** Gray opaque flat colonies, raised elevation, 2-3mm diameter, generally circular but tending to elongate in the direction of spreading, ground glass appearance and a rough, fimbriate edge. Lecithinase negative. Incubation longer than 48hrs may result in a lighter gray or white centre to the colony. Phenolic odour due to the production of p-cresol. Colonies fluoresce yellow-green under UV light. Confirm by latex agglutination.

**References**

Brazier J.S. (1993) Rôle of the Laboratory in Investigations of Clostridium difficile Diarrhoea. Clinical Infectious Diseases 16 (4) 228-33.

# *Brilliant Green Agar (modified)*

***(Phenol Red Brilliant Green Agar, BPLS)***

**LAB034**

**Description**

First introduced by Kristensen *et al* in 1925 as a selective medium for the isolation of salmonellae (except *S. typhi*). The medium was modified by the Netherlands Institute for Public Health, Utrecht. The modification was to increase the selectivity of the medium by increasing the dye concentration. This formulation is quoted by the International Standards Organisation, standard European Community Methods, the American Public Health Association and the Association of Official Analytical Chemists. The medium is suitable for subcultures from selective enrichment media. However because this medium is highly selective, small numbers of salmonellae may be missed. This medium is definitely not recommended for *S. typhi* and *Shigella* spp. Less inhibitory media such as X.L.D. and Hektoen Enteric Agar will be useful in detecting salmonellae and shigellae inhibited by Brilliant Green Agar.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 5.0 |
| Balanced Peptone No. 1 | 10.0 |
| Yeast Extract | 3.0 |
| Disodium hydrogen phosphate | 1.0 |
| Sodium dihydrogen phosphate | 0.6 |
| Lactose | 10.0 |
| Sucrose | 10.0 |
| Phenol red | 0.09 |
| Brilliant green | 0.0047 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 52 grams of powder and disperse in 1 litre of deionised water. Allow to soak for ten minutes and then bring to the boil with frequent swirling to dissolve the solids and cool to 47˚C in a water bath. Pour plates and dry the surface before inoculation. DO NOT remelt or autoclave: overheating causes precipitation of the medium. Store plates away from light.

**Appearance:** Red/Brown, clear gel.

**pH:** 6.9 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface streaking for single colonies, a heavy inoculum can be used.

**Incubation:** 37˚C for 18-24 hours aerobically.

**Growth characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Salmonella* spp. | 1-1.5 | CV.E.G. | Pink colonies | (red zone in medium) |
| *S. typhi* | 1.0 | CV.E.G. | Pink/Red | (may not grow) |
| *E. coli* | no growth | | | (0.5-1.0 yellow colony) |
| *Proteus* spp | no growth | | | |
| *Enterococcus* spp. | no growth | | | |
| *S. sonnei* | no growth | | | |

**References**

Edel, W. and Kamplemacher, E.H. (1968). Comparative studies on Salmonella isolation in eight European laboratories. Bull. Wld. Hlth. Org. 39: 487-491.

Edel, W. and Kamplemacher, E.H. (1969). *Salmonella* infections in nine European laboratories using a standard technique. Bull Wld. Hlth. Org. 41: 297-306.

American Public Health Association (1966). Recommended Methods for the Microbiological Examination of Foods, 2nd end. (ed. J.M. Sharf) A.P.H.A. Washington.

Association of Official Analytical Chemists (AOAC) (1978) Bacteriological Analytical Manual, 5th edn., Washington D.C.

Pharmacopoeia of culture media for food microbiology. (1987). Int. J. Food Microbiol. 513: 245-247. 3/112

# *Brilliant Green Bile 2% Broth*

**LAB051**

**Description**

A modification of MacConkey's medium, formulated in 1926 by Dunham and Schoenlein, for the recovery of coliform bacteria in foodstuffs and water. The brilliant green and bile inhibit most Gram positive organisms thus overcoming the problem of some *Clostridium* spp. fermenting lactose and giving false positive results.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 10.0 |
| Lactose | 10.0 |
| Ox Bile | 20.0 |
| Brilliant green | 0.0133 |

**Method for reconstitution**

Weigh 40 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then warm to dissolve. Dispense into tubes or bottles with inverted Durham tubes. Sterilise by autoclaving at 115˚C for 15 minutes.

**Appearance:** Green, clear.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. WDCM 00031
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 1 month at 2-8˚C in the dark.

**Inoculation:** Serial 1:10 dilutions of homogenised sample are inoculated into the broth in the proportion of 1ml sample to 9ml broth. Ensure the Durham tube is free from gas bubbles before commencing inoculation. B.G.B. broth can be used at double strength if required but cannot be sterilised by autoclaving, pasteurisation must be used instead.

**Incubation:** *E. coli* and thermotrophs 44˚C for 18 hours aerobically. Mesopholic coliforms 32˚C for 24-48 hours aerobically. Psychrotrophic coliforms 4˚C for 10 days aerobically.

**Interpretation:** Turbidity, colour changes (to yellow or yellowish green) and production of gas are all presumptive evidence of the growth of organisms of the coli-aerogenes group. Confirmation by indole production in Tryptone Water LAB129 (44˚C for *E. coli*).

**References**


Pharmacopoeia of Culture Media for Food Microbiology (1987). Int. J. Food Microbiol. 5:3:206-207.

American Public Health Association, American Water Works Association and Water Pollution Control Federation, (1975), Standard Methods for the Examination of Water and Wastewater, 14th ed., Washington D.C.

Association of Official Analytical Chemists (AOAC). Bacteriological Analytical Manual, 5th ed., Washington, D.C. Association of Official Analytical Chemists. 1978.

Hausler, W. J. (ED) (1972). Standard Methods for the Examination of Dairy Products. 13th ed., Washington. D.C. American Public Health Association.

Shane, M.S. (1947). Studies on false confirmed test using B.G.B. and comparison studies on Lauryl Sulfate Tryptose Broth as presumptive medium. J. Am. Water Works Assoc., 39: (4), 337.


# *Bromocresol Purple Lactose Agar*

***(Drigalski agar)***

**LAB121**

**Description**

A non-selective differential medium for the isolation and enumeration of *Enterobacteriaceae* from urine, water and food products. Lactose fermenting organisms produce yellow colonies, non lactose fermenters produce purple colonies.

| Typical Formula | g/litre |
|---|---|
| Peptone mixture | 7.4 |
| Lactose | 8.5 |
| Bromocresol purple | 0.025 |
| Agar No. 1 | 12.0 |

**Method for reconstitution**

Weigh 28 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 47˚C then pour into Petri dishes.

**Appearance:** Purple, clear agar.

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms: *E. coli.* WDCM 00013
*S. aureus* WDCM 00034

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, plating either over entire surface for colony count or streak out to single colonies.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *E. coli* | 1.5-2.0 | CV.E.G. | Yellow | (N.L.F. – purple) |
| *Proteus* spp. | 1.0-1.5 | CV.E.G. | Purple | |
| *Salmonella* spp. | 1.0-2.0 | CV.E.G. | Purple | |
| *S. aureus* | 0.5 | CV.E.G. | Cream | (purple if N.L.F.) |
| *E. faecalis* | 0.5 | CV.E.G. | Yellow | |

**References**


Drigalski, C. (1902). Uber ein Verfahren zum Nachweis der Typhusbacillen. Z. Hyg. Infekt. 39:283-300.

## *Buffered Listeria Enrichment Broth*

**LAB139**

**Description**

A medium for the selective enrichment of food and environmental samples for *Listeria* spp, LAB139 is a buffered version of the 'FDA' broth LAB138. The extra buffering capacity maintains the pH of the enrichment culture during incubation, ensuring optimum conditions for the recovery of *Listeria* spp.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 17.0 |
| Soy peptone | 3.0 |
| Sodium chloride | 5.0 |
| Dipotassium hydrogen phosphate | 2.5 |
| Glucose | 2.5 |
| Yeast Extract | 6.0 |
| Potassium dihydrogen phosphate | 1.35 |
| Disodium hydrogen phosphate | 9.6 |

**Method for reconstitution**

Weigh 47 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of X139 reconstituted in 50% alcohol. Aseptically dispense into sterile tubes or bottles.

**Appearance:** Yellow, clear.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *L. monocytogenes* WDCM 00021
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 14 days at 2-8˚C in the dark.

**Inoculation:** Add 25 grams of sample to 225mls of Buffered Listeria Enrichment Broth and homogenise.

**Incubation:** 30˚C aerobically for up to 48 hours.

**Subculture:** After 24 and 48 hours onto Listeria Isolation Medium – LAB122.

## *Buffered Peptone Water*

**LAB046**

**Description**

A pre-enrichment medium designed to help sublethally damaged salmonellae recover before introducing them into a selective medium. This nutrient medium is free from inhibitors and is well buffered to maintain the pH at 7.2 for the incubation period. Sublethal injury to salmonellae occurs in many food processes and this pre-enrichment step greatly increases recovery of these organisms.

| Typical Formula | g/litre |
|---|---|
| Peptone | 10.0 |
| Sodium chloride | 5.0 |
| Disodium hydrogen phosphate | 3.7 |
| Potassium dihydrogen phosphate | 1.5 |

**Method for reconstitution**

Weigh 20 grams of powder and disperse in 1 litre of deionised water. Mix to dissolve then distribute into tubes or bottles. Sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Pale straw, clear liquid.

Minimum Q.C. organisms: *E. coli* WDCM 00013

**pH:** 7.2 ± 0.2

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Add 25 grams of sample to 225ml of Buffered Peptone Water and homogenise.

**Incubation:** Aerobically at 37˚C for 18-24 hours.

**Subculture:** 10ml aliquots in 100ml of Selenite Cystine Broth LAB055 and 0.1ml into 10ml Rappaport Vassiliadis Medium LAB086.

**References**

Edel W. and Kampelmacher E.H. (1973). Bull. Wld Hlth Org. 48: 167-174.

Poemla P.K. and Silliker J.H. (1976) *Salmonella* in Compendium of Methods for microbiological examination of foods. Am. Pub. Health Ass., Washington.

## *Buffered Peptone Water (ISO)*

**LAB204**

**Description**

Formulated to ISO 6579, this pre-enrichment medium is designed to help sublethally damaged salmonellae recover before introducing them into a selective medium. This nutrient medium is free from inhibitors and is well buffered to maintain pH 7.0 for the incubation period. Sublethal injury to salmonellae occurs in many food processes and this pre-enrichment step greatly increases recovery of these organisms.

| Typical Formula | g/litre |
|---|---|
| Enzymatic digest of casein | 10.0 |
| Sodium chloride | 5.0 |
| Disodium hydrogen phosphate (anhydrous) | 3.6* |
| Potassium dihydrogen phosphate | 1.5 |

*Equivalent to 9.0g of disodium hydrogen phosphate dodecahydrate

**Method for reconstitution**

Weigh 20.1 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then distribute into tubes or bottles. Sterilise by autoclaving for 15 minutes at 121ºC.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: pale straw, clear liquid

**pH:** 7.0 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Staphylococcus aureus* WDCM 00034
*Salmonella typhimurium* WDCM 00031
*Listeria monocytogenes* WDCM 00021

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight

Prepared media: capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Add 25 grams of sample to 225ml of Buffered Peptone Water and homogenise.

**Incubation:** Aerobically at 37°C for 18-24 hours.

**References**

BS EN ISO 6579:2002 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. (Incorporating Corrigendum No. 1)

# *Campylobacter Blood Free Selective Medium*

***(Modified CCDA-Improved)***

**LAB112**

**Description**

A blood free medium which will support the growth of most enteric campylobacters. The selective cocktail X112 (or X212) makes the medium selective for *C. jejuni.* and *C. coli* when incubated at 37˚C. With this product incubation at 42˚C is no longer necessary and higher recovery rates have been reported at 37˚C than at 42˚C.

The supplement X112 (or X212) consists of cefoperazone and amphotericin and is superior to the selective cocktails of Skirrow, Butzler and Blazer-Wang all of which contain antibiotics shown to be inhibitors to *C. coli.* The colonial morphologies of *Campylobacter* spp. on this medium are distinctive.

| Typical Formula | g/litre |
|---|---|
| Peptone blend | 25.0 |
| Bacteriological Charcoal | 4.0 |
| Sodium chloride | 3.0 |
| Sodium desoxycholate | 1.0 |
| Ferrous sulphate | 0.25 |
| Sodium pyruvate | 0.25 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 45.5 grams of powder, disperse in 1 litre of deionised water and allow to soak for 10 minutes. Swirl to mix, then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C then add 2 vials of X112 supplement, mix well and pour into Petri dishes. Continuously mix whilst pouring to prevent the charcoal settling.

**Appearance:** Black agar.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *C. jejuni*
*E. coli* (inhibition) WDCM 00013
*Candida albicans* (inhibition)

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** *C. jejuni, C. coli.* surface streaking to single colonies.

**Incubation:** 37˚C for 48 hours in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. *C. cinaedi* and *C. fennelliae* require up to 7 days.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *C. jejuni* | 2.0-3.0 | F.E.G. | Grey/ White | Efflorescent (spreading moist) |
| *C. coli* | 1.0-2.5 | CV.E.G. | Creamy Grey | Moist |

LAB112

**References**


Bolton F.J. Hutchinson D.N., Parker G. Reassessment of Selective Agars and Filtration Techniques for Isolation of Campylobacter Species from Feces. Eur.J. Clin. Microbiol. Infects. Dis. (1988) 7 p 155-160.

Bolton F. J. (1988) Personal Communication.

Bolton F.J. Hutchinson D.N., Parker G. Isolation of Campylobacter: What are we missing? J.Clin.Path. (1987) 40 p 702-703.

Goosens H., De. Boeck M., Coignau H., Vlaes L., Van Den Borre C., Butzler J.P. Modified Selective Medium for Isolation of Campylobacter spp from Feces: Comparison with Preston Medium, a Blood Free Medium, and a Filtration System. J.Clin. Micro. (1986) 24 p 840-843.

Gun-Munro J., Rennie R.P., Thornley J.H. Richardson H.L., Hodge D., Lynch J. Laboratory and Clinical Evaluation of Isolation Media for Campylobacter jejuni J. Clin Micro. (1987). 25 p 2274-2277.

Herbert G.A., Hollis D.G., Weaver R.E., Karmali M.A., Simor A.E., Roscoe M., Fleming P.C., Smith, S.S. Lane J. Evaluation of a Blood-Free, Charcoal-Based, Selective Medium for the Isolation of Campylobacter organisms from Faeces. J. Clin. Micro. (1986) 23 p 456-459.


# *Campylobacter Enrichment Broth*

***(Bolton Formulation)***

**LAB135**

**Description**

A selective enrichment broth for the isolation of *Campylobacter* spp. from food, environmental samples and faeces. The use of a selective enrichment broth enhances the recovery of sub-lethally damaged organisms due to processing of foods, or if small numbers of campylobacters are present in heavily contaminated specimens. This broth has been shown to give appreciably better results than Preston Broth.

| Typical Formula | g/litre |
|---|---|
| Meat Peptone | 10.0 |
| Lactalbumin Hydrolsates | 5.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 5.0 |
| Haemin | 10.0mg |
| Sodium pyruvate | 0.5 |
| α – ketoglutaric acid | 1.0 |
| Sodium metabisulphite | 0.5 |
| Sodium carbonate | 0.6 |

**Method for reconstitution**

Weigh 27.6 grams of powder, disperse in 1 litre of deionised water and allow to soak for 10 minutes. Swirl to mix and autoclave at 121˚C for 15 minutes. Cool to 47˚C, add 2 vials of selective supplement X132 reconstituted with 5ml of 50% alcohol and 50ml of saponin lysed horse blood, mix well and dispense into sterile containers.

**Appearance:** Translucent, wine-red with a fine black suspension.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *Campylobacter jejuni*
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Capped containers: 7 days at 2-8˚C in the dark.

**Inoculation:** Food homogenate is added to broth in a ratio of 1:4 (w/v) in screw cap containers leaving a head space of 1.5 cm. For faeces 1ml of a 10% suspension in Buffered Peptone Water LAB046 is added to 5ml of broth.

**Incubation:** Aerobically at 37˚C for 2-4 hours, followed by a further 16-44 hours at 42˚C.

**Subculture:** Onto Campylobacter Blood Free Selective Medium LAB112.

**References**

Bolton, F.J. Personal Communication.

Hunt J.M., Abeyta C., and Tran T. (1998) Chapter 7 Campylobacter in FDA Bacteriological Analytical Manual 8th Edition.

## *Cary-Blair Medium*

**LAB505**

**Description**

Cary-Blair medium is a transport medium for the collection and shipment of clinical specimens based on the formulation of Cary and Blair. The low nutrient content of the medium and the inclusion of phosphate buffer prevents bacterial overgrowth by *E. coli*, *Citrobacter freundii* and *Klebsiella aerogenes*, which can occur in other transport medium containing sodium glycerophosphate. The low oxidation-reduction potential of the medium ensures bacterial survival over long periods.

Cary and Blair reported recovery of cholera vibrios up to 22 days, *Salmonella* and *Shigella* after 49 days and *Yersinia pestis* up to 75 days storage at 28°C. Survival of *Vibrio parahaemolyticus* has been reported after a 35-day period at 70-80°F.

The medium may be prepared as a pre-reduced anaerobic sterilised medium (PRAS) by the Holdeman and Moore method.

| Typical Formula | g/litre |
|---|---|
| Disodium hydrogen phosphate | 1.1 |
| Sodium thioglycollate | 1.5 |
| Sodium chloride | 5.0 |
| Calcium chloride | 0.09 |
| Agar | 5.6 |

**Method for reconstitution**

Weigh 13.3 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix then bring to the boil, with mixing, to dissolve the agar. Distribute into bijou bottles and sterilise by immersing in free-steam for 15 minutes. Allow the medium to cool and tighten the screw caps to prevent water loss.

For transport of fastidious anaerobic bacteria prepare the medium as directed and fill into long narrow screw capped tubes, or to the neck of the Bijou bottle.

**Appearance:** Colourless soft gel.

**pH:** 8.4 ± 0.2

Minimum Q.C. organisms: *Shigella sonnei* ATCC 25931
*Vibrio furnissi* NCTC 11218

**Storage of Prepared Medium:** Store away from light at 2-8°C or at room temperature (22-25°C) for up to 19 months.

**Inoculation:** Use sterile, cotton-tipped swab on wooden sticks to collect the specimen. Push the swab down one third of the depth of the medium and cut the stick. Screw the cap firmly on the bottle. Label the bottle and send to the testing laboratory without delay.

**References**

Cary, S.G. and Blair, E.B. (1964). J. Bact. 88. 96-98.

Cary, S.G., Matthew, M.S., Fusillo, M.H., and Harkins, C. (1965). Survival of *Shigella* and *Salmonella* in a new transport medium for shipment of clinical samples. Am. J. Clin, Path. 43. 294-296.

Crookes, E.M. and Stuart, R.D. (1959) J. Pathol. Bacteriol. 78. 283-288.

Stuart, R.D. (1959) Public Health Reports 74. 431-438.

Neumann D.A., Benenson, M.W., Hubster, E. and Tuan, N.T.N. (1971). Am. J. Clin. Path. 57.

Wren, M.W.D. J. Med. Microbiol. 10. 195-201.

Holdeman, L.V. and Moore, W.E.C (1975) Anaerobe Laboratory Manual, Virginia Polytechnic Institute Anaerobe Laboratory, 3rd Ed.

## *C.E.M.O. Agar Base*

***(Contagious Equine Metritis Organism)***

**LAB078**

**Description**

This medium is a selective isolation medium for *Taylorella equigenitalis* the causative organism of contagious equine metritis. The medium is a sugar free base with a mixture of high grade casein and soy peptones as nutrients and with L-cystine and sodium sulphite as supplements and reducing agents. The medium is made selective with the addition of amphotericin (5 mg/L) and trimethoprim (10 mg/L). Streptomycin (200 mg/L) can also be used but sensitive variants of *T. equigenitalis* have been described.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 15.0 |
| Soy Peptone | 5.0 |
| Sodium chloride | 5.0 |
| Agar No. 2 | 12.0 |
| L-Cystine | 0.3 |
| Sodium sulphite | 0.2 |

**Method for reconstitution**

Weigh 37.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 80˚C, add 50ml of sterile horse blood and allow to 'chocolate'. Further cool to 47˚C before adding antibiotic selective agents. Mix well and pour into Petri dishes.

**Appearance:** Chocolated Blood Agar.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *H. equigenitalis*

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking out for single colonies.

**Incubation:** 37˚C in 10% $CO_2$ for 2-3 days.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *T. equigenitalis* | 0.1-1.0 | CV.E.G. | Cream | colony size variation is common |

**References**

Atherton, J.G. (1978). Inhibition of the C.E.M. organism in mixed cultures. Vet. Rec. 432.

Mackintosh, M.E. (1981). Bacteriological techniques in the diagnosis of equine genital infections. Vet. Rec. 108, 52-55. Atherton, J.G. Personal Communication.

Fleming, M.P. Tribe. G. W. (1977). Vet. Rec. 101, 1470.

# *Cetrimide Agar (USP/EP/JP)*

**HP010**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the isolation and identification of *Pseudomonas aeruginosa*, in non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. Gelatin is a source of nitrogen whilst glycerol acts as a carbon source. Cetrimide is a quarternary ammonium compound that inhibits the growth of a wide range of Gram-positive and some Gram-negative micro-organisms. Magnesium chloride and dipotassium sulphate improve the production of pyoverdin and pyocyanin pigments that combine to give *Pseudomonas aeruginosa* characteristic green colonies. According to the Harmonised European Pharmacopoeia, subculture is carried out onto the medium after enrichment in Casein Soya Bean Digest Broth.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of gelatin | 20.0 |
| Magnesium chloride | 1.4 |
| Dipotassium sulphate | 10.0 |
| Cetrimide | 0.3 |
| Agar | 13.6 |

**Method for reconstitution**

Disperse 45.3 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, add 10mL of glycerol, swirl to mix and boil to dissolve. Sterilise by autoclaving atg121ºC for 15 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: translucent, pale straw gel

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Pseudomonas aeruginosa* ATCC 9027
*Escherichia coli* ATCC 8739

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the European Pharmacopoeia 8.0 subculture is performed from enrichment in casein soya bean digest broth onto the agar surface.

**Incubation:**

Incubate at 30-35°C for 18-72 hours.

**Growth Characteristics**

| Organism | Shape & surface | Colour | Other |
|---|---|---|---|
| *P. aeruginosa* | CV.E.G. | Yellow/green | Fluorescent under UV light |

**References**

European Pharmacopoeia 8th Edition

# *C.L.E.D. Medium*

***(Mackey and Sandys)***
***(Cystine Lactose Electrolyte Deficient-Single Indicator)***

**LAB041**

**Description**

A medium for urine culture first described by Mackey and Sandys in 1960. The absence of electrolytes inhibits the swarming of *Proteus* spp. Cystine is added for the benefit of those organisms which have a specific cystine requirement. Differentiation of lactose and non lactose fermenters is achieved using bromothymol blue as pH indicator. This medium supports the growth of fastidious organisms that do not require blood.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 4.0 |
| Beef Extract | 3.0 |
| Tryptone | 4.0 |
| Lactose | 10.0 |
| L-Cystine | 0.128 |
| Bromothymol blue indicator | 0.02 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 36 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C mix and distribute into Petri dishes.

**Appearance:** Green/blue clear gel.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*S. aureus* WDCM 00034

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface inoculation either spreading for single colonies or spread evenly over entire surface for colony counts.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *E. coli* | 2.0-3.0 | CV.E.G. | Yellow | (blue if non lactose fermenters) |
| *Proteus* spp. | 2.0-3.0 | CV.E.G. | Blue | |
| *Salmonella* spp. | 2.0-3.0 | CV.E.G. | Blue | (yellow if lactose +ve) |
| *Staph. aureus* | 1.0-1.5 | CV.E.G. | Yellow | (blue if non-lactose fermenting) |
| other *Staphylococcus* spp. | 0.5-1.5 | CV.E.G. | Blue-white | (yellow if lactose fermenting) |
| *Enterococcus* spp. | 0.5 | CV.E.G. | Yellow | |

**References**

Mackey, J.P. and Sandys, G.H. (1966). Diagnosis of urinary infections. Brit.Med.J. 1: 1173.

Guttman, D and Naylor, G.R.E. (1967). Dip-slide: an aid to quantitative urine culture in general practice. Brit.Med. J. 3: 343-345.

# *C.L.E.D. Medium (Bevis modification)*

***(Cystine Lactose Electrolyte Deficient – Double Indicator)***

**LAB006**

**Description**

Bevis modified Mackey and Sandys original medium by introducing a double indicator to improve the differentiation of lactose and non lactose fermenting coliforms, staphylococci and streptococci. The swarming of *Proteus* spp. is inhibited. Lab M C.L.E.D. will grow many of the more demanding streptococci of Lancefield groups A, B, C, G and F. This medium may not grow *Pasteurella* spp. or halophilic organisms.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 4.0 |
| Beef Extract | 3.0 |
| Tryptone | 4.0 |
| Lactose | 10.0 |
| L-Cystine | 0.128 |
| Bromothymol blue indicator | 0.02 |
| Andrade's indicator | 0.08 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 36 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C and mix before pouring.

**Appearance:** Green/blue, clear gel.

**pH:** 7.5 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*S. aureus* WDCM 00034

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation method:** Surface inoculation, either streaking for single colonies or spread evenly over entire surface for colony counts.

**Incubation:** 37˚C for 24 hours aerobically.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *E. coli* | 2.0-3.0 | CV.E.G. | Yellow/ Orange | (Blue if non-lactose fermenter) |
| *Proteus* spp. | 2.0-3.0 | CV.E.G. | Blue | |
| *Salmonella* spp. | 2.0-3.0 | CV.E.G. | Blue | (Yellow-orange if lactose +ve) |
| *S. aureus* | 1.0-1.5 | CV.E.G. | Yellow/ Orange | (Blue if non-lactose fermenting) |
| Other staphylococci | 0.5-1.5 | CV.E.G. | Blue-White | (Yellow if lactose fermenting) |
| *Enterococcus* spp. | 0.5 | CV.E.G. | Yellow-Orange | |

**References**


Bevis, T.D. (1968). A modified electrolyte-deficient culture medium. J. Med. Lab. Tech., 25: 38-41.

Mackey, J.P. and Sandys, G.H. (1966). Diagnosis of urinary infections, Brit.Med. J., 1: 1173.

Sandys, G.H. (1960). A new medium for preventing swarming of *Proteus* spp. with a description of a new medium suitable for use in routine laboratory practice. J. Med.Lab. Tech., 17: 224-233.


# *Columbia Agar Base*

**LAB001**

**Description**

A general purpose nutritious agar base formulated by Ellner *et al*. When further enriched by the addition of sterile blood, Columbia agar can be used for the isolation of most clinically significant pathogens. The blood can be 'chocolated' if required. The medium can be made selective for various groups by the addition of appropriate antibiotic mixtures eg:

Streptococci – Colistin/Oxolinic acid (X013)
*Gardnerella* spp. – Colistin/Nalidixic acid (X011)
*C. perfringens* – Neomycin (X015) (X016)
Campylobacters - (X214)
Staphylococci/streptococci – Colistin/Naladixic acid (X012)

| Typical Formula | g/litre |
|---|---|
| Columbia Peptone Mixture | 23.0 |
| Corn Starch | 1.0 |
| Sodium chloride | 5.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 41 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 48˚C and add 5-7% sterile, defibrinated horse or sheep blood. Mix well before pouring.

**Appearance:** Cherry red if blood is fresh and well oxygenated.

Minimum Q.C. organisms:
*S.pyogenes* NCTC 8198
*E.coli* ATCC 8739
*S.aureus* ATCC 6538

**pH:** 7.3 ± 0.2

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface plating, streaking out for single colonies.

**Incubation:** 37˚C aerobically or microaerobically for 24 hours. Anaerobically for 24 and 48 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. aureus* | 1.5-2.0 | CV.E.G. | White-Yellow | Haemolytic |
| *S. pyogenes* | 0.5-1.0 | CV.E.G.(D) | White | α, β-haemolytic dependent on strain |
| *S. pneumoniae* | 0.5-1.5 | F.E.G. | Grey | greenish discolouration in medium, mucoid in $H_2/C0_2$ |
| *Neisseria meningitidis* | 1.0-2.0 | CV.E.G. | trans/ Grey | (mucoid) |
| *E. coli* | 2.0-3.0 | CV.E.G. | Opaque/ Grey | (haemolytic) |
| *Ps. aeruginosa* | 0.5-4.0 | F.CR.D. | Opaque Grey | many colonial forms (green pigment) (haemolytic) (mucoid) |
| *C. perfringens* | 1.5-2.0 | CV.CR.G. | Grey | usually target haemolysis (non haemolytic) |
| *B. fragilis* | 1.0-1.5 | CV.E.G. | Grey | (mucoid) |
| *P. anaerobius* | P.P.-0.5 | CV.E.G. | White/ Grey | |

**References**

Ellner, P.D., Stoessel, C.J., Drakeford, E and Vasi, F. (1966). A new culture medium for medical bacteriology. Amer. J. Clin Pathol., 45:502-504.

Goldberg, R.L., and Washington, J.A., (1976). Comparison of isolation of *Haemophilus vaginalis (Corynebacterium vaginale)* from Peptone-Starch-Dextrose Agar and Columbia Colistin-Nalidixic Acid Agar. J. Clin. Microbiol., 4:245-247.

Thayer, D.D. and Martin, H. E. (1966). An improved medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. Publ. Hlth. Report, 81:559-562.

## *Columbia II Agar Base*

**LAB215**

**Description**

A modification of the original Columbia Agar base formulation, Columbia II Agar Base provides a medium that is suitable for use with both defibrinated horse and defibrinated sheep blood.

Originally described as a general purpose nutritious agar base by Ellner *et al*. Columbia Agar is more frequently used when enriched by the addition of sterile blood. The medium is suitable for supporting the growth of a variety of microorganisms including the majority of clinically significant pathogens.

| Typical Formula | g/litre |
|---|---|
| Columbia peptone mixture | 25.1 |
| Soluble starch | 1.0 |
| Sodium chloride | 5.0 |
| Agar | 12.0 |
| **Grams per litre** | **43.1** |

**Method for reconstitution**

Disperse 43.1 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise by autoclaving at 121oC for 15 minutes. Cool to 47°C and add 5-7% sterile defibrinated horse or sheep blood. Mix well before dispensing into sterile Petri dishes. Dry the agar surface before use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: Opaque red gel (with blood)

**pH:** 7.3 ± 0.2

**Hazard classification**
NR – Not regulated

Minimum Q.C. organisms:
*S.pyogenes* NCTC 8198
*E.coli* ATCC 8739
*S.aureus* ATCC 6538

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C

**Inoculation:** Surface plating, streaking out for single colonies.

**Incubation:** Incubate for 24-48 hours at 30°C or 3°C in an aerobic, anaerobic, $CO_2$ or microaerophilic environment as requried for growth of target organism.

**References**

Ellner, P.D., Stoessel, C.J., Drakeford, E. and Vasi, F. (1966). A new culturemedium for medical bacteriology. *Amer J. Clin Pathol.* **45.** 502-504.

## *CSEB - Cronobacter sakazakii Enrichment Broth*

***Modified Lauryl Sulphate Tryptose Broth Vancomycin Medium***

**LAB081**

**Description**

*Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) is a member of the *Enterobacteriaceae* family and has been associated with serious outbreak infections in neonates (premature infants) which have been fed on infant formula milk. Although rarely causing infections in immunocompetent adults, *C. sakazakii* has been implicated in sepsis, meningitis and necrotising enterocolitis with a high death rate in neonates. This opportunistic pathogen is common in the environment and its ability to survive desiccation presents a significant risk for post pasteurisation contamination and survival in spray dried milk products.

Based on lauryl sulphate tryptose broth, Cronobacter sakazakii Enrichment Broth (CSEB) has added sodium chloride for extra selectivity against competing organisms. The antibiotic vancomycin is also added to inhibit Gram-positive organisms such as *Staphylococccus aureus* which may be able to grow in this medium.

| Typical Formula | g/litre |
|---|---|
| Enzymatic digest of animal and plant tissue | 20.0 |
| Lactose | 5.0 |
| Sodium chloride | 34.0 |
| Dipotassium hydrogen phosphate | 2.75 |
| Potassium dihydrogen orthophosphate | 2.75 |
| Sodium lauryl sulphate | 0.1 |
| **Grams per litre** | **64.6** |

**Method for reconstitution**

Weigh 64.6 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and if required, heat gently to dissolve. Dispense in 10ml volumes and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C.

Prepare a solution of vancomycin in distilled water at a concentration of 1mg/ml. Add 0.1ml of the vancomycin solution to the sterile broth to obtain a final concentration of 0.1mg per 10ml (10mg/L) of CSEB.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid

**pH:** 6.8 ± 0.2

**Hazard classification**
NR – Not regulated

Minimum Q.C. organisms:
*Cronobacter sakazakii* ATCC 12868
*Cronobacter muytjensii* ATCC 51329
*Escherichia coli* ATCC 25922 (inhibition)

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media (with vancomycin): 1 day at 2-8°C in the dark..

**Inoculation:** Following pre-enrichment in Buffered Peptone Water, transfer 0.1mL of the obtained culture into 10ml LAB081 CSEB.

**Incubation:** Incubate at 44°C + 0.5°C for 24 hours + 2 hours.

**Sub-culture & Interpretation:** After incubation, tubes showing turbidity should be streaked onto HAL012 CSIM (ISO).

**References**

Bowen AB, Braden CR (2006). "Invasive Enterobacter sakazakii disease in infants". *Emerging Infect Dis* **12** (8): 1185–9.

Caubilla-Barron J & Forsythe S (2007). "Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae* in dehydrated infant formula". *Journal Food Protection* **13**: 467-472.

"Enterobacter sakazakii infections associated with the use of powdered infant formula--Tennessee, 2001" (2002). *MMWR Morb Mortal Wkly Rep* **51** (14): 297–300.

Farmer JJ III, Asbury MA, Hickman FW, Brenner DJ, the Enterobacteriaceae Study Group (USA) (1980). "*Enterobacter sakazakii*: a new species of "Enterobacteriaceae" isolated from clinical specimens". *Int J Syst Bacteriol* **30**: 569–84.

ISO/TS 22964:2006(E) Milk and milk products – Detection of *Enterobacter sakazakii*.

Iversen C, Lehner A, Mullane N, *et al* (2007). "The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter genomospecies* 1". *BMC Evol Biol* **7**: 64.

Iversen C, Mullane N, Barbara McCardell, *et al* (2008). "*Cronobacter* gen. nov., a new genus to accommodate the biogroups of Enterobacter sakazakii, and proposal of Cronobacter sakazakii gen. nov. comb. nov., C. malonaticus sp. nov., C. turicensis sp. nov., C. muytjensii sp. nov., C. dublinensis sp. nov., Cronobacter genomospecies 1, and of three subspecies, C. dublinensis sp. nov. subsp. dublinensis subsp. nov., C. dublinensis sp. nov. subsp. lausannensis subsp. nov., and C. dublinensis sp. nov. subsp. lactaridi subsp. nov.". IJSEM.

Lai KK (2001). "*Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature". *Medicine (Baltimore)* **80** (2): 113–22.


# *D.C.A.*

***(Desoxycholate Citrate Agar)***

**LAB029**

**Description**

This is Leifson's original formulation of this selective medium for the isolation of *Salmonella* spp. and *Shigella* spp. from faeces and environmental samples. It has approximately half the quantity of inhibitors used in the Hynes modification. The medium uses sodium citrate and sodium desoxycholate as inhibitors. Sodium thiosulphate is the substrate for the enzyme thiosulphate reductase being broken down to form sulphite and hydrogen sulphide. The hydrogen sulphide reacts with the ferric ions to produce a black precipitate of ferrous sulphide. This gives a typical black centre to the colonies of most species of *Salmonella.*

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 5.0 |
| Balanced Peptone No. 1 | 5.0 |
| Lactose | 10.0 |
| Sodium citrate | 5.0 |
| Sodium thiosulphate | 5.0 |
| Ferric citrate | 1.0 |
| Sodium desoxycholate | 2.5 |
| Neutral Red | 0.025 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 45.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then bring to the boil with frequent stirring. When the medium boils up into the neck of the flask, quickly remove from the source of heat and allow the froth to subside. Return to the heat and allow the foam to boil up into the neck of the flask once more. Remove at once and cool to 47˚C approx. before pouring plates. Dry the surface before inoculation. DO NOT REMELT OR AUTOCLAVE THIS MEDIUM.

**Appearance:** Pale pink, translucent, a fine precipitate of desoxycholate may be present which may clear if the pH is increased by the growth of organisms.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. WDCM 00031
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking for single colonies.

**Incubation:** 37˚C for 18-24 hours aerobically.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. typhi* | 0.5. 1.5 | CV.E.G. | transp Yellow | (black centre) |
| Other *Salmonella* spp. | 1.5-2.0 | CV.E.G | transp (Opaque) Yellow | (black centre) (clearing around colony) |
| *S. sonnei* | 1.5-2.0 | CV.E.G. | transp (pinkish) | (more opaque centre) |
| *E. coli* | P.P.-1.5 (uninhibited) | CV.E.D. (G) | Red/Pink | ppt in medium |
| *Citrobacter* spp. | P.P.-2.0 | CV.E.D. (G) | Red/Pink | ppt in medium (black centre) |
| *Proteus* spp. | 1.0-2.0 | CV.E.G. | Yellow | (black/grey centre) fishy odour (clearing around colony) |


**References**

Hynes. M. (1942). The isolation of intestinal pathogens by selective media. J. Path. Bact. 54. 193-207.

Liefson. E. (1935). New culture media based on Sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Path. Bact. 40: 581-589.


# *D.C.A. Hynes*

***(Desoxycholate Citrate Agar -Hyne's modification)***

**LAB065**

**Description**

This modification of Leifson's D.C.A. medium was introduced in 1942. The medium was designed to be more inhibitory to commensal flora whilst allowing for adequate growth of *Salmonella* spp and *Shigella* spp. The citrate and desoxycholate levels are significantly increased. To keep the desoxycholate in solution the pH also had to be increased. The medium still uses lactose fermentation and hydrogen sulphide production as differential indicators.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 5.0 |
| Balanced Peptone No. 1 | 5.0 |
| Lactose | 10.0 |
| Sodium thiosulphate | 5.4 |
| Sodium citrate | 8.5 |
| Ferric citrate | 1.0 |
| Sodium desoxycholate | 5.0 |
| Neutral red | 0.02 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 52 grams of powder, disperse in 1 litre of deionised water in a two litre flask. Bring to the boil over a gauze, swirling frequently to prevent burning. Simmer for 30 seconds to dissolve. Cool to 47˚C before pouring plates. Dry the surface before inoculation. DO NOT REMELT OR AUTOCLAVE THIS MEDIUM.

**Appearance:** Pink, clear, bile aggregates may appear on the surface on refrigeration.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. WDCM 00031
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking out for single colonies.

**Incubation:** 37˚C aerobically for 24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. sonnei* | 1.0-2.0 | CV.E.G.(D) | Colourless - pale pink | |
| *Salmonella* spp. | 1.0-4.0 | CV.E.G. | Colourless | (black centre) |
| *S. typhi* | 0.5-1.5 | CV.E.G. | Colourless | (Black/grey centre) |
| *E. coli* | P.P.-1.5 | CV.CR.D. | Red | (No growth) |
| *Proteus* spp | 0.5-2.0 | CV.E.G. | Colourless | (Yellow) fishy odour |

**References**

Hynes, M. (1942). The isolation of intestinal pathogens by selective media. J. Path. Bact, 54: 193-207

## *D.C.L.S. Agar*

***(Desoxycholate Citrate Lactose Sucrose Agar)***

**LAB003**

**Description**

A modification of Leifson's D.C.A. medium which incorporates sucrose as an additional fermentable substrate to differentiate lactose negative sucrose positive coliforms from *Salmonella* spp. This medium is unsuitable for the isolation of *Yersinia* spp. which are sucrose positive.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 7.0 |
| Beef Extract | 3.0 |
| Lactose | 5.0 |
| Sucrose | 5.0 |
| Sodium citrate | 10.5 |
| Sodium thiosulphate | 5.0 |
| Sodium desoxycholate | 2.5 |
| Agar No. 2 | 12.0 |
| Neutral Red | 0.03 |

**Method for reconstitution**

Weigh 50 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes then heat gently with frequent mixing and bring to the boil. Simmer for 1 minute to complete dissolution of the solids. Cool to 47˚C then distribute 20ml into 90mm Petri dishes. Dry the surface by partial exposure, before use. DO NOT REMELT OR AUTOCLAVE THIS MEDIUM.

**Appearance:** Pale Pink, clear.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *Salmonella typhimurium* WDCM 00031
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface plating, streaking out to single colonies.

**Incubation:** 37˚C aerobically for 24 hours

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. typhi* | 0.5-1.0 | CV.E.G. | Trans. colourless | |
| Other *Salmonella* spp. | 1.5-2.0 | CV.E.G. | Slight cloudy colourless | |
| *S. sonnei* | 1.5-2.0 | CV.E.G | Trans. Pinkish | (More opaque centre) |
| *E. coli* | P.P.-1.5 (inhibited) | CV.E.G.(D) | Red | (ppt around colonies) |
| *Citro*bacter spp | P.P.-2.0 (inhibited) | CV.E.D.(G) | Red | (ppt around colonies) |
| *Proteus* spp. | 1.0-2.0 | CV.E.G. | Yellow | (Fishy odour) |

**References**

Hynes, M. (1942). The isolation of intestinal pathogens by selective media. J. Path. Bact. 54: 193-207.

Leifson, E. (1935). New culture media based on sodium desoxycholate for the isolation of colon bacilli in milk and water. J. Path. Bact. 40: 581-589.

## *D/E Neutralising Agar*

***(Dey & Engley)***

**LAB188**

**Description**

D/E Neutralising Agar is used to neutralise and determine the bactericidal activity of antiseptics and disinfectants. Developed by Dey and Engley, this agar neutralises a broad spectrum of antimicrobial chemicals, producing better results than those obtained using alternatives such as Letheen Agar. Complete neutralisation is required to prevent false results arising from disinfectant carryover. D/E Neutralising Agar is used as the plating medium when testing disinfectants using D/E Neutralising Broth and D/E Neutralising Broth Base. It can also be used to test disinfectants by a disc diffusion method. D/E Neutralising Agar contains thioglycollate to neutralise mercurial compounds, sodium thiosulphate to neutralise iodine and chlorine and sodium bisulphite to neutralise formaldehyde and gluteraldehyde. Lecithin is included to neutralise quaternary ammonium compounds and Polysorbate 80 neutralises phenols, hexachlorophene, formalin, and combined with lecithin, ethanol. Bromocresol purple allows detection of growth via a colour change from purple to yellow when organisms ferment the glucose contained in the medium.

| Typical Formula | g/litre |
|---|---|
| Glucose | 10.0 |
| Lecithin | 7.0 |
| Sodium thiosulphate | 6.0 |
| Polysorbate 80 | 5.0 |
| Tryptone | 5.0 |
| Sodium bisulphite | 2.5 |
| Yeast extract | 2.5 |
| Sodium thioglycollate | 1.0 |
| Bromocresol purple | 0.02 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 54.0 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes and allow to set.

**Appearance:** Purple opaque gel.

**pH:** 7.6 ± 0.2

Minimum Q.C. organisms:
*Bacillus subtilis* WDCM 00070
*Escherichia coli* WDCM 00013
*Pseudomonas aeruginosa* WDCM 00025
*Salmonella typhimurium* WDCM 00031
*Staphylococcus aureus* WDCM 00034

**Storage of Powder:** Store at 2-8°C in the dark. Formulation is very hygroscopic, keep container tightly closed after use.

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C in the dark.

**Inoculation:** Consult appropriate references as this product is used in several procedures.

**Incubation:** 37°C aerobically for 24-48 hours.

**Interpretation:** Count all colonies for total counts, count yellow colonies for differential acid producer count. Non-acid producing colonies are grey to colourless.

**References**

Roberts, D., Hooper, W. and Greenwood, M., (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.

Engley Jr., F.B. and Dey, B.P. (1970). A universal neutralising medium for antimicrobial chemicals. Presented at the Chemical Specialities Manufacturing Association (CSMA) Proceedings, 56th Mid Year Meeting.

Dey, B.P. and Engley Jr., F.B. (1995). Comparison of Dey and Engley
(D/E) Neutralising medium to Lethhen medium and Standards
Methods Medium for recovery of *Staphylococcus aureus* from sanitised surfaces. J. Ind. Microbiol. 14:21-25.

Curry, A.S., Graf, J.G. and McEwen Jr., G.N. (ed.) (1993) CFTA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

# *D/E Neutralising Broth*

***(Dey & Engley)***

**LAB187**

**Description**

D/E Neutralising Broth is used to neutralise and determine the bacteriocidal activity of antiseptics and disinfectants. Developed by Dey and Engley, D/E Neutralising Broth neutralises a broad spectrum of antimicrobial chemicals, producing better results than those obtained using alternatives such as Letheen Broth, Thioglycollate Medium and Neutralising Buffer. Complete neutralisation is required to prevent false results arising from disinfectant carryover. When used with D/E Neutralising Broth Base the action of the antimicrobial agent can be assessed, i.e. whether it is bacteriostatic or has bactericidal properties. The procedure is based upon D/E Neutralising Broth Base being deficient of all neutralising agents, therefore the potency of the disinfectant is not diminished after addition to the medium. Whereas, when disinfectant is added to the D/E Neutralising Broth, its activity is neutralised allowing for the detection of any bacteria presence. D/E Neutralising Broth contains thioglycollate to neutralise mercurial compounds, sodium thiosulphate to neutralise iodine and chlorine and sodium bisulphite to neutralise formaldehyde and gluteraldehyde. Lecithin is included to neutralise quaternary ammonium compounds and Polysorbate 80 neutralises phenols, hexachlorophene, formalin, and combined with lecithin, ethanol. Bromocresol purple allows detection of growth via a colour change from purple to yellow when organisms ferment the glucose contained in the medium.

| Typical Formula | g/litre |
|---|---|
| Glucose | 10.0 |
| Lecithin | 7.0 |
| Sodium thiosulphate | 6.0 |
| Polysorbate 80 | 5.0 |
| Tryptone | 5.0 |
| Sodium bisulphite | 2.5 |
| Yeast extract | 2.5 |
| Sodium thioglycollate | 1.0 |
| Bromocresol purple | 0.02 |

**Method for reconstitution**

Weigh 39.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Purple opaque liquid.

**pH:** 7.6 ± 0.2

Minimum Q.C. organisms:
*Bacillus subtilis* WDCM 00070
*Escherichia coli* WDCM 00013
*Pseudomonas aeruginosa* WDCM 00025
*Salmonella typhimurium* WDCM 00031
*Staphylococcus aureus* WDCM 00034

**Storage of Powder:** Store at 2-8°C in the dark. The formulation is very hygroscopic therefore keep the container tightly closed after use.

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Consult appropriate references as this product is used in several procedures.

**Incubation:** 37°C aerobically for 24-48 hours.

**Interpretation:** Examine all tubes for increased turbidity, formation of a pellicle or a colour change from purple to yellow, indicating bacterial growth.

**References**

Roberts D., Hooper, W. and Greenwood, M., (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.

Engley Jr., F.B. and Dey, B.P. (1970). A universal neutralising medium for antimicrobial chemicals. Presented at the Chemical Specialities Manufacturing Association (CSMA) Proceedings, 56th Mid Year Meeting.

Dey, B.P. and Engley Jr., F.B. (1995). Comparison of Dey and Engley
(D/E) Neutralising medium to Lethhen medium and Standards Methods Medium for recovery of *Staphylococcus aureus* from sanitised surfaces. J. Ind. Microbiol. 14:21-25.

Curry, A.S., Graf, J.G. and McEwen Jr., G.N. (ed.) (1993) CFTA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

## *D/E Neutralising Broth Base*

***(Dey & Engley)***

**LAB186**

**Description**

D.E. Neutralising Broth Base is a nutritious medium deficient of all neutralising agents. Therefore when a test disinfectant is added to the broth, the potency is undiminished. Developed for use with Dey and Engley's Neutralising Broth (LAB187), incorporating D.E. Neutralising Broth Base into the test procedure allows the user to differentiate between bacteriostatic and bactericidal activity, and to detect viable organisms that remain after treatment. Its use is recommended in disinfectant evaluation, environmental sampling and water-miscible cosmetics in accordance with Cosmetic, Toiletry and Fragrance Association (CTFA) guidelines..

| **Typical Formula** | **g/litre** |
|---|---|
| Glucose | 10.0 |
| Tryptone | 5.0 |
| Yeast extract | 2.5 |
| Bromocresol purple | 0.02 |
| **Grams per litre** | **17.5** |

**Method for reconstitution**

Weigh 17.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving for 15 minutes at 121°C.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, purple liquid

**pH:** 7.6 ± 0.2

Minimum Q.C. organisms:
*Bacillus subtilis* WDCM 00070
*Escherichia coli* WDCM 00013
*Pseudomonas aeruginosa* WDCM 00025
*Salmonella typhimurium* WDCM 00031
*Staphylococcus aureus* WDCM 00034

**Storage of Powder:** 10-25°C away from direct sunlight.

**Storage of Prepared Medium:** in capped containers for up to 3 months at 15-20°C in the dark.

**Inoculation:** Consult appropriate references as this product is used in several procedures.

**Incubation:** 37°C aerobically for 24-48 hours.

**Interpretation:** Examine all tubes for turbidity, indicating growth.

**References**

Roberts, D., Hooper, W. and Greenwood, M., (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.

Engley Jr., F.B. and Dey, B.P. (1970). A universal neutralising medium for antimicrobial chemicals. Presented at the Chemical Specialities Manufacturing Association (CSMA) Proceedings, 56th Mid Year Meeting.

Dey, B.P. and Engley Jr., F.B. (1995). Comparison of Dey and Engley (D/E) Neutralising medium to Lethhen medium and Standards Methods Medium for recovery of *Staphylococcus aureus* from sanitised surfaces. J. Ind. Microbiol. 14:21-25.

Curry, A.S., Graf, J.G. and McEwen Jr., G.N. (ed.) (1993) CFTA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C..

## *Dermatophyte Test Medium (D.T.M.)*

**LAB117**

**Description**

A modification of the formulation of Taplin, Zaias, Rebell and Blank for the detection of dermatophytic fungi. This medium helps in the differentiation between saprophytic and environmental fungi.

| **Typical Formula** | **g/litre** |
|---|---|
| Balanced Peptone No. 1 | 10.0 |
| Glucose | 40.0 |
| Agar No. 2 | 12.0 |
| Phenol Red | 0.2 |

**Method for reconstitution**

Weigh 62 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes then bring to the boil with frequent stirring. Dissolve 2 vials of Chloramphenicol X009 (or 1 vial X209) in ethanol and add these to the agar, mix well and distribute into tubes or universal containers. Sterilise at 121°C for 15 minutes, allow to cool in the sloped position.

**Note:** Do not exceed the times stated for sterilisation, overheated acidified agar loses gel strength and the sugars are caramelised.

**Appearance:** Orange, clear gel.

**pH:** 5.5 ± 0.2

Minimum Q.C. organisms: *Aspergillus* spp. NCIMB 50097
*Trichophyton* spp.

**Storage of Prepared Medium:** Slopes – up to 1 month at 2-8°C in the dark.

**Inoculation:** Surface plating or stab inoculation.

**Incubation:** 22-25°C aerobically for 10-14 days.

**Interpretation:** Dermatophytes appear as fluffy colonies, colour varies with species, the medium is reddened. Fungi other than dermatophytes cause the medium to become yellow due to acid production. If incubation is prolonged the medium may become reddened. Yeasts appear as white creamy colonies. Blastomyces, Histoplasma and Coccidiodes may also turn the medium red, though these are rarely encountered in lesions associated with ring worm.

**References**

Taplin, D., Zaias, N., Rebell, G., Blank, H. (1969). Isolation and recognition of dermatophytes on a new medium. (DTM) Arch. Dermatol. 99: 203-209.

# *Dextrose Tryptone Agar*

**LAB020**

**Description**

A medium for the enumeration of thermophilic spore bearers in foods. The medium was designed to detect the thermophilic bacteria causing 'flat sour'spoilage of canned foods. The medium also detects the 'flat sour' organism *Bacillus stearothermophilus* in sugar and other sweetening agents used in the preparation of frozen dairy foods, cereals and other food products.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Glucose | 5.0 |
| Bromocresol purple | 0.04 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**
Weigh 27 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil to dissolve agar before dispensing in 20ml amounts for poured plate technique. Sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Purple clear agar.

**pH:** 6.9 ± 0.2

Minimum Q.C. organisms: *B. stearothermophilus*

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Pour plate technique, pre-heat sample by steaming for 20 minutes if a spore count is required.

**Incubation:** For Thermophiles – Aerobically for 48 hours at 55˚C. For Mesophiles – Aerobically for 48-72 hours at 30-32˚C.

**Interpretation:** Count all colonies for total counts, count yellow colonies for differential acid producer count. Non acid producing colonies are grey to colourless.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *B. stearothermophilus* | 2.0 | Rz.D | Yellow zone mauve centre |
| *Bacillus* spp. | 1.5-3.0 | Rz.D | Mauve (Yellow halo) |
| *S. aureus* | 0.5-1.5 | CV.E.G. | Yellow |
| *E. coli* | 1.0-1.5 | CV.E.G. | Yellow |
| *Klebsiella* spp. | 1.5-2.5 | CV.E.G. | Yellow (mucoid) |
| *Enterococci* | 0.5 | CV.E.G. | Yellow |
| *Proteus* spp. | 2.0-3.0 | RzD | Yellow (spreads) |

**References**


Williams, O.B. (1963). Tryptone Medium for the Detection of Flat Sour Spores. Food Research 1, (3): 217-221.

American Public Health Association. (1972). Standard Methods for the Examination of Dairy Products. 13th Edn. Ed. W.J. Hausler A.P.H.A. Washington.

Tanner, F.W. (1946). The Microbiology of Food 2nd edn., Garrard Press, Champners.

Baumgartner, J.G. and Hersom, A.C. (1956). Canned Foods. 4th Edn. Churchill, London.


# *DG18 Agar*

***Dichloran (18%) Glycerol Agar***

**LAB218**

**Description**

Lab M's Dichloran 18% Glycerol Agar (DG18 Agar) is a medium for the enumeration of osmophilic yeasts and xerophilic moulds in food and animal products.

DG18 Agar is used for the enumeration of viable osmophilic yeasts and xerophilic moulds in food or animal feed products with a water activity of less than or equal to 0.95 by a colony count technique. This includes such foods as dry fruits, jams, cakes, dried meat, salted fish, grains, cereals, flours, nuts, spices, condiments and some animal feeds. This medium is not suitable for the examination of dehydrated products with a water activity of less than or equal to 0.60 and does not allow the enumeration of mould spores or the detection of halophilic xerophilic fungi found in dried fish.

The reduction in water activity in this medium is achieved by the addition of glycerol at approximately 18% and this is very important as many yeast and moulds actually require a low water activity to enhance growth and colony development. The medium also contains the antifungal agent dichloran, which restricts the spreading of mucoraceous fungi and restricts the colony size of other genera making colony counting an easier task.

Additional selectivity against bacterial growth is achieved by the incorporation of the heat-stable antibiotic Chloramphenicol. Glucose is incorporated as the fermentable carbohydrate source, with casein enzymatic digest providing the essential vitamins, minerals, amino acids, nitrogen and carbon.

Developed with reference to ISO 21527-2:2008, this medium is tested to the performance requirements of the standard.

| Typical Formula | g/litre |
|---|---|
| Casein enzymatic digest | 5.0 |
| D-Glucose | 10.0 |
| Potassium dihydrogen phosphate | 1.0 |
| Magnesium sulphate | 0.5 |
| Dichloran | 0.002 |
| Chloramphenicol | 0.1 |
| Agar | 15.0 |
| **Grams per litre** | **31.6** |

**Method for reconstitution**
Disperse 31.6g of powder in 1 litre of distilled water. Allow to soak for 10 minutes and swirl to mix. Add 220g Glycerol and if necessary, heat gently to dissolve. Sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw gel

**pH:** 5.6 ± 0.2

**Hazard classification**
T – Toxic

Minimum Q.C. organisms:
*Saccharomyces cerevisiae* WDCM 00058
*Escherichia coli* WDCM 00013 (inhibited)

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation (as per ISO 21527-2:2008):** Inoculate plates in duplicate with 0.1ml of test sample. Spread the liquid over the agar surface using a sterile spreader until the liquid is completely absorbed.

**Incubation (as per ISO 21527-2:2008):** Incubate aerobically with lids uppermost at 25°C ± 1°C for 5-7 days.

**Interpretation (as per ISO 21527-2:2008):** Read plates between 2 – 5 days. Select dishes containing less than 150 colonies/propagules and count these colonies/propagules.

If fast-growing moulds are a problem, count colonies/propagules after 2 days and again after 5-7 days of incubation.

**References**

Bell, C., Neaves, P., Williams, A.P. (2005). Food microbiology and laboratory practice. Blackwell, Oxford. p324.

Beuchat, L.R. Media for detecting and enumerating yeasts and moulds. In Corry, J.E.L., Curtis, GDW., Baird, R.M., Editors. Handbook of Culture Media for Food Microbiology, p369-386.

Beuchat LR, Frandberg E, Deak T, Alzamora SM, Chen J, Guerrero AS, López-Malo A, Ohlsson I, Olsen M, Peinado JM, Schnurer J, de Siloniz MI, Tornai-Lehoczki J. (2001). Performance of mycological media in enumerating desiccated food spoilage yeasts: an interlaboratory study. *Int. J. Food Microbiol*. Oct 22;**70**(1-2):89-96.

BS ISO 21257-2:2008 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds. Part 2: Colony count technique in products with water activity less than or equal to 0,95.

Deak, T., Chen. J., Golden D.A., Tapia, M.S., Tornai-Lehoczki, J., Viljoen, B.C., Wyder, M.T. Beuchat, L.R. (2001). Comparison of dichloran 18% glycerol (DG18) agar with general purpose mycological media for enumerating food spoilage yeasts. *Int. J. Food Microbiol.* **67**, 49-53.

Hocking, A.D., Pitt, J.I. (1980). Dichloran-glycerol based medium for the enumeration of xerophilic fungi form low moisture foods. *Appl. Environ. Microbiol.* **39**, 488-492.

# *Diagnostic Semi-Solid Salmonella Agar (Diassalm)*

***According to Van Netten and Van der Zee et al***

**LAB537**

**Description**

Diassalm, as developed by Van Netten *et al* (1991), is a semi-solid differential medium for the isolation of *Salmonella* spp. from food and water. It is an improved modification of MSRV (De Smedt and Bolderdijk 1988) and SR (Perales and Audicana 1989) with regard to the composition of the basal medium, selective system and the introduction of a differential system.

The original basal medium was a commercially available sulphide mobility-indole medium (SIM BBL) (Blazevic 1968). Lab M have substituted their raw materials into Blazevic's formula to create a richer base for Diassalm. Selectivity is achieved by the use of malachite green oxalate, magnesium chloride and novobiocin. The diagnostic properties of Diassalm are based on the use of two indicator systems; saccharose combined with bromocresol purple; and ferro-iron in combination with thiosulphate.

The efficiency of Diassalm is due to the ability of salmonellae to move through the highly selective mobility medium in a Petri dish, whilst the double diagnostic system allows visualisation of motile and non-motile suspected salmonellae due to blacking zones against the turquoise background. Diassalm can be seeded after pre-enrichment or after 8hr enrichment in selective broth (De Smedt and Bolderdijk 1987).

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Meat Peptone | 6.1 |
| Ferrous ammonium sulphate | 0.2 |
| Sodium thiosulphate | 5.0 |
| Sucrose | 7.5 |
| Lactose | 0.5 |
| Bromocresol purple | 0.08 |
| Malachite green oxalate | 0.037 |
| Magnesium chloride anhyd. | 11.0 |
| Agar No.1 | 2.8 |

**Method for reconstitution**

Weigh 53.0 grams of powder, disperse in 1 litre of deionised water. Mix well, bring quickly to the boil. Allow to cool to 47˚C and add 1 vial of Novobiocin supplement – X150 (10mg/vial). Mix well and pour plates. Nitrofurantoin may be used instead of Novobiocin to improve the isolation of *S. enteritidis.*

**Appearance**:- Green transparent, soft gel

**pH:** 5.5 ± 0.2

Minimum QC organisms:
*Salmonella typhimurium* WDCM 00031
*Escherichia coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days: at 2-8˚C in the dark.

**Inoculation:** 3 drops (0.1ml) of 8 to 20hr. incubated pre-enrichment broth are inoculated in one spot in the centre of one plate of Diassalm.

**Incubation:** At 42 ± 0.5˚C or 37˚C for 18-24 hours. Keep the lid uppermost at all times.

**Interpretation**

After incubation the plates are examined for a mobility zone with a purple/black colour change. When the mobility zone is absent, but the centre is blackened, non-motile salmonellae may be present. A loopfull of the motile zone which is the farthest from the sample inoculum (or the blackened centre if non-motile) is sub-cultured onto brilliant green agar and XLD agar. Futher biochemical and serological identification are performed according to recognised procedure.

Direct latex agglutination may also be carried out from the edge of the mobility zone.

**References**

Blazevics, D.J. (1968) Appl. Microbiol. 16, 688

De Smedt J.M. *et al* 1987 J. Food Protection 50, 658

Perales, I and Audicana. Evaluation of semi-solid Rappaport medium for detection of Salmonellae in meat products. J. Food Protection 52,

Van Netten, P., Van de Moosdijk, A., Perales, I. and Mossel, D.A.A. Letters in Applied Microbiology

Van Netten, P., Van der Zee, H., and Van der Moosdijk, A., (1991). The use of diagnostic selective semi-solid medium for the isolation of *Salmonella* enteritidis from poultry. Proceedings of the 10th Symposium on the quality of poultry meat, Spelderholt Beckbergen, pp. 59-67.

Van der Zee, H., and Van Netten, P., (1992). Diagnostic semi-solid media based on Rappaport-Vassiliadis Broth for the detection of *Salmonella* spp. and *S. enteritidis* in foods. Proceedings of the International Symposium of *Salmonella* and Salmonellosis.

Van der Zee, H., (1992). Detection of *Salmonella* spp. with the use of a standard method, diagnostic semi-solid agars and immunocapture kit. Proceedings Third World Congress Foodborne infections and intoxications, Berlin.

# *DN'ase Agar*

**LAB095**

**Description**

DN'ase agar provides a convenient means of identifying potentially pathogenic staphylococci, based on the ability of coagulase-positive species to split DNA. DN'ases produced by the organisms hydrolyse the DNA molecule to a mixture of smaller mono and poly nucleotides. DiSalvo observed perfect correlation between coagulase activity and DN'ase production using *S. aureus* strains from clinical specimens. Other publications have also reported a close correlation.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Deoxyribonucleic acid (DNA) | 2.0 |
| Sodium chloride | 5.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 39 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 47˚C then pour into Petri dishes.

**Appearance:** Pale cream, clear.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCIMB 50080
*S. epidermidis* NCIMB 50082

**Storage of Prepared Medium:** Plates – up to 7 days: at 2-8˚C in the dark. Capped container – up to 1 month at 4˚C in the dark.

**Inoculation:** Use a heavy inoculum on a small area. Four or more organisms can be tested on one 90mm Petri dish.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Interpretation:**

Having obtained good growth flood the plate with 1N hydrochloric acid. This will precipitate the DNA in the medium. DN'ase producing organisms will be surrounded by a clear area where the DNA has been broken down into fractions which are not precipitated by the Hydrochloric acid. Gram positive, catalase positive cocci that produce DN'ase can be provisionally classified as *S. aureus,* and confirmed by tube coagluase or thermostable DN'ase tests. DN.'ase is also produced by some Gram negative bacilli such as *Serratia marcescens, Pseudomonas aeruginosa.* Some corynebacteria and streptococci may also produce DN'ase.

**References**


Baird-Parker, A. C. 1965. The classification of staphylococci and micrococci from world-wide sources. J. Gen. Microbiol. 38, 363-387. Black, W. A., Hodgson, R. and McKechnie, A. 1971.

DiSalvo, J. W. 1958 Deoxyribunuclease and coagulase activity of micrococci. Med. Tech. Bull. U.S. Armed Forces Med. J. 9, 191.

Martin, W. J and Ewing, W. H. 1967. The deoxryibonuclease test as applied to certain gram-negative bacteria. Can. J. Microbiol. 13, 616-618.

Messinova, O. V., Yusupova, D. V. and Shamsutdinov, N. S. 1963. Deoxyribonuclease activity of Corynebacterium and its relation to virulence. Fed. Proc. 22, T1033.

Streitfeld, M. M., Hoffmann, E. M. and Janklow, H. M. 1962. Evaluation of extracellular deoxyribonuclease activity in Pseudomonas. J. Bacteriol. 84, 77. Wannamaker, L. W. 1964. Streptococcal deoxryribonuclease, pp. 140-165. J. W. Uhr (ed.). The Streptococcus, Rheumatic Fever, Glomerulophritis. Baltimore: Williams & Williams.

Weckman, B. G. and Catlin, B. W. 1957 Deoxryribonuclease activity of micrococci from clinical sources. J. Bacteriol. 73, 747-753.

Zierdt, C. H. and Golde, D. W. 1970. Deoxryribonuclease-positive Staphylococcus epidermidis strains. Appl. Microbiol. 20(1), 54-57.


# *DRBC Agar*

***Dichloran Rose Bengal Chloramphenicol Agar***

**LAB217**

**Description**

Lab M's Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) is a medium for the enumeration of yeasts and moulds in food and animal products.

Developed with reference to ISO 21527-1:2008, this medium is tested to the performance requirements of the standard.

Used for the enumeration of viable yeasts and moulds in products with a water activity of greater than 0.95 such as eggs, meat, some dairy products, fresh pastes, fruit and vegetables, DRBC Agar is designed to suppress the colonial growth of 'spreader' moulds and in doing so allow easier performance of the colony count technique on yeasts and moulds.

The use of the anti-fungal agent, dichloran, restricts spreading of mucoraceous fungi and restricts the colony size of other genera. Rose bengal also assists in the reduction of colony sizes and is selective against bacteria.

Additional selectivity against bacterial growth is achieved by the incorporation of the heat-stable antibiotic Chloramphenicol. Glucose is incorporated as the fermentable carbohydrate source, with an enzymatic digest of animal & plant tissues providing the essential vitamins, minerals, amino acids, nitrogen and carbon.

| Typical Formula | g/litre |
|---|---|
| Enzymatic digest of animal & plant tissues | 5.0 |
| D-Glucose | 10.0 |
| Potassium dihydrogen phosphate | 1.0 |
| Magnesium sulphate | 0.5 |
| Dichloran | 0.002 |
| Chloramphenicol | 0.1 |
| Rose bengal | 0.025 |
| Agar | 15.0 |
| **Grams per litre** | **31.7** |

**Method for reconstitution**

Disperse 31.7g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, pink

Finished medium: clear, pink gel

**pH:** 5.6 ± 0.2

**Hazard classification**

T – Toxic

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation (as per ISO 21527-1:2008):** Inoculate plates in duplicate with 0.1ml of test sample. Spread the liquid over the agar surface using a sterile spreader until the liquid is completely absorbed.

**Incubation (as per ISO 21527-1:2008):** Incubate aerobically with lids uppermost at 25°C ± 1°C for 5 days.

**Interpretation (as per ISO 21527-1:2008):** Read plates between 2 – 5 days. Select dishes containing less than 150 colonies/propagules and count these colonies/propagules.

If necessary, use a magnifier to distinguish between cells of yeasts or moulds and bacteria from colonies.

**References**

Bacteriological Analytical Manual, 8th edition, Revision A, 1998. Chapter 18 Yeasts, Molds and Mycotoxins. Authors: Valerie Tournas, Michael E. Stack, Philip B. Mislivec, Herbert A. Koch and Ruth Bandler. Revised: 2000-APR-17

Beuchat and Cousin (2001). In Downes and Ito (ed.). Compendium of Methods for the Microbiological Examination of Foods, 4th edition. American Public Health Association.

BS ISO 21257-1:2008 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds. Part 1: Colony count technique in products with water activity greater than 0.95.

King Jr, A.D., Hocking, A.D. and Pitt, J.I. (1979). Dichloran-Rose Bengal Medium for Enumeration and Isolation of Molds from Foods. *J. Appl. Environ. Microbiol.* 1979, **37**, 959-964.

# *DRCM (ISO)*

***Differential Reinforced Clostridial Medium (ISO)***

**LAB220**

**Description**

Differential Reinforced Clostridial Medium ISO (DRCM) is a medium for the detection and enumeration of the spores of sulphite-reducing anaerobes as described in BS EN 26461-1.

Sulphite reducing anaerobes, in particular clostridia, can be indicators of remote and intermittent pollution. Widespread in the environment, being found in human and animal faeces, soil and waste water, the spores are more resistant to physical and chemical factors than vegetative cells and able to survive for long periods in water. The spores may also be resistant to chlorination at the levels commonly used in water treatment.

DRCM has been developed for use with the Most Probable Number (MPN) method to determine the MPN of anaerobes (Clostridia) per volume of sample. The formulation includes peptone, yeast extract, meat extract, starch & L-cysteine for nutrition with glucose providing the energy source. Sodium acetate provides partial selectivity.

Clostridia are able to reduce sulphite to sulphide – forming iron sulphide. Iron (III) citrate is included in the formulation as an indicator of sulphite reduction. Blackening in the medium indicates that iron sulphide has been formed and therefore that sulphite reduction has occurred.

Other bacteria are able to form sulphide, so vegetative cells must be first be removed from the test sample by an appropriate process e.g. heat treatment.

| **Typical Formula** | **g/litre** |
|---|---|
| Peptone mix | 10.0 |
| Yeast extract | 1.5 |
| Starch | 1.0 |
| Hydrated sodium acetate | 5.0 |
| Glucose | 1.0 |
| L-Cysteine hydrochloride | 0.5 |
| Sodium sulphite | 0.4 |
| Iron (III) citrate | 0.7 |
| **Grams per litre** | **30.1** |

**Method for reconstitution**

Disperse 30.1g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving for 15 minutes at 121°C.

Medium should be used on day of preparation. If medium is stored, tubes should be reheated to deoxygenate the medium. Tubes should not be reheated more than once.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid

**pH:** 7.1 ± 0.2

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation (as per BS EN 26461-1:1993):** Before the test, the sample of water should be heated in a water bath at 75 ± 5°C for 15 minutes from the time it reaches that temperature.

Add 50ml of sample to 50ml double strength medium (x5).
Add 10ml of sample to 10ml double-strength medium (x5).
Add 1ml of sample to 25ml single-strength medium (x5).

If required add 1ml of a 1 in 10 dilution of the sample to 25ml single-strength medium (x5).

To qualitatively examine 100ml drinking/bottled water without performing MPN, add 100ml sample to 100ml double-strength medium.

If required, top up all bottles with single-strength medium to bring the volume of liquid level with the neck of the bottle, and to ensure that only a very small volume of air remains. Seal the bottles hermetically, or incubate under anaerobic conditions.

**Incubation (as per BS EN 26461-1:1993):** Incubate aerobically with lids uppermost at 37°C ± 1°C for 44 ± 4 hours.

Large volumes of culture in hermetically sealed glass bottles may explode due to gas production. The addition of iron wire, heated to redness and placed into the medium before inoculation, may aid anaerobiosis

**Interpretation (as per BS EN 26461-1:1993):** Bottles in which blackening is observed, as a result of the reduction of sulphite and the precipitation of iron (II) sulphide, shall be regarded as positive.

**References**

BS EN 26461-1:1993 / BS 6068-4.8:1993 / ISO 6461-1:1986. Water quality – Detection and enumeration of he spores of sulfite-reducing anaerobes (clostridia) – Part 1: Method by enrichment in a liquid medium.

Freame, B. & Fitzpatrick, B.W.F. (1967). The use of Differential Reinforced Clostridial Medium for the isolation and enumeration of Clostridia from foods. *The Society for Applied Microbiology Technical Series n. 5: Isolation of Anaerobes,* ed. Shapton, D.A. & Board, R.G. **Vol. 5.** London Academic Press. 49-55.

Gibbs, M.B. (1973). The detection of Clostridium welchii in the Differential Reinforced Clostridial Medium technique. *J. Appl. Bact.* **36**. 23-33.

# *EC Medium*

***(Escherichia coli Medium)***

**LAB171**

**Description**

EC Medium (*Escherichia coli* Medium) is a selective enrichment broth designed for the isolation of coliforms, including *E. coli*, from water and food samples. It was the recommended medium of the American Public Health Association (APHA) and the AOAC.

EC Medium is made selective for coliforms by the inclusion of Bile Salts No.3 in the dehydrated medium. The selective nature of this medium ensures that the growth of non-coliform bacteria is minimised. The medium is buffered by the addition of potassium phosphates and osmotically balanced by sodium chloride. The medium is used at 37°C for coliform organisms and 45.5°C is recommended for the isolation *E. coli.*

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Lactose | 5.0 |
| $K_2HPO_4$ | 4.0 |
| $KH_2PO_4$ | 1.5 |
| Sodium chloride | 5.0 |
| Bile Salts No. 3 | 1.5 |

**Method for reconstitution**

Weigh 37.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix. Dispense into tubes of appropriate volume and, where applicable, add Durham tubes. Sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Clear straw broth.

**pH:** 6.9 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Enterococcus faecalis* WDCM 00087 (inhibition)
*Bacillus subtilis* WDCM 00070 (inhibition)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Coliforms: Follow the methods and procedures as stated in Standard Methods for the Examination of Water and Wastewater and Compendium of Methods for the Microbiological Examination of Foods.

**Incubation:** 45.5°C for 18-24 hours aerobically for *E. coli* and 37°C for 18-24 hours, aerobically for coliforms.

**Interpretation:** Turbidity of broth and gas collection in the Durham tube indicates the presumptive growth of organisms from the coli-aerogenes group. All broths should be sub-cultured onto selective media whether turbid or not.

**References**

American Public health Association, (1980). Standards Methods for the Examination of Water and Wastewater, 15th Edition, American Public Health Association, Inc., Washington, D.C.

American Public health Association, (1976). Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Inc., Washington, D.C.

Association of Official Analytical chemists. (1995). Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Perry and Hajna, (1943). American Journal of Public Health, 33:550.

Perry and Hajna, (1944). American Journal of Public Health, 34:735.

# *E.E. Broth*

***(Enterobacteriaceae Enrichment Broth)***

**LAB091**

**Description**

E.E. Broth is recommended as an enrichment medium when examining food and feedstuffs for *Enterobacteriaceae.* It is a modification of LAB051 Brilliant Green Bile Broth, with an improved buffering capacity to encourage early growth and prevent autosterilization. E.E. Broth uses glucose instead of lactose to make the medium a test for all enterobacteria including non lactose fermenting organisms.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 10.0 |
| Dextrose | 5.0 |
| Disodium hydrogen phosphate | 6.45 |
| Potassium dihydrogen phosphate | 2.0 |
| Bile Salts | 20.0 |
| Brilliant green | 0.0135 |

**Method for reconstitution**

Weigh 43.5 grams of powder and add to 1 litre of deionised water. Swirl to dissolve, warm gently if necessary, then distribute into bottles or tubes and heat at 100˚C for 30 minutes only. Cool rapidly. OVERHEATING THIS MEDIUM WILL ADVERSELY AFFECT ITS PERFORMANCE.

**Appearance:** Green, clear.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*B. subtilis* WDCM 00070 (inhibition)

**Storage of Prepared Medium:** capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Add 1 part of sample suspension or dilution to 10 parts of medium.

**Incubation:** 44˚C for 18 hours for thermotrophs. 32˚C for 24-48 hours for mesotrophs. 4˚C for 10 days for psychrotrophs.

**Interpretation:** Turbidity and a colour change to yellow-green is presumptive evidence of *Enterobacteriaceae.* Subculture onto confirmatory media e.g. LAB088 V.R.B.G.A. must be carried out.

**References**

Mossel, D. A. A., Visser, M. and Cornelissen, A. M. R. 1963. The examination of foods for *Enterobacteriaceae* using a test of the type generally adopted for the detection of salmonellae. J.Appl. Bacteriol. 26, 444-452.

Mossel, D. A. E., Harrewijn, G. A. and Nesselrooy-van Zadelhoff, C. F. M. 1974. Standardisation of the selective inhibitory effect of surface active compounds used in media for the detection of *Enterobacteriaceae* in food and water. Health Lab. Sci. 11, 260-267. Richard, N. 1982. Monitoring the quality of selective liquid media by the official French dilution technique used for the bacteriological examination of foods. In: Quality assurance and quality control of microbiological culture media, edited by J. E. L. Corry, G.I.T.-Verlag Darmstadt, pp. 51-57.

# *Endo Agar*

**LAB060**

**Description**

This medium was developed in 1914 for the isolation of *Salmonella typhi;* other media have since proved superior for this purpose, but Endo Agar has a role as a coliform medium. It is recommended by the American Public Health Association as a standard medium for the enumeration of coliforms in water and dairy products. In this medium acetaldehyde is produced by coliforms and then fixed by the sulphite to produce a metallic sheen with the basic fuchsin dye. Most enteric Gram negative organisms will grow well, whilst Gram positive organisms are mostly inhibited.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 10.0 |
| Lactose | 10.0 |
| Dipotassium phosphate | 3.5 |
| Sodium sulphite | 2.5 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 41 grams of powder, disperse in 1 litre of deionised water. Add 4ml of a 10% w/v alcoholic solution of basic fuchsins (95% ethyl alcohol). Bring to the boil with frequent swirling to dissolve the solids. Sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C in a water bath before pouring. The precipitate typically associated with this medium should be dispersed by gentle swirling prior to pouring the plates.

This medium is light sensitive and should therefore be stored in the dark, preferably under refrigeration. The medium will become dark red in colour if exposed to light.

**Basic Fuchsin is a potential Carcinogen and care should be taken when handling it to avoid inhalation of the powdered dye and contamination of the skin.**

**Appearance:** Pale pink/orange

**pH:** 7.5 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking out for single colonies.

**Incubation:** 37˚C for 18-48 hours aerobically.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *E. coli* | 1.0-2.0 | CV.E.G. | Deep Red | (Metallic sheen) |
| *K. aerogenes* | 1.0-2.5 | CV.E.G. | Red | (mucoid) |
| *Proteus* spp | 2.0-3.0 | CV.E.G. | Pale Pink colourless | |
| Gram positive organisms | no growth. | | | |

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking out for single colonies.

**Incubation:** 37˚C for 18-48 hours aerobically.

**References**


Endo, 1914, Centr. Bakt., Abt 1, Orig., 35: 109.

American Public Health Association, 1975. Standard Methods for the Examination of Water and Wastewater, 14th Edn. American Public Health Association, Inc. Washington D.C.

American Public Health Association, 1972. Standard Methods for the Examination of Dairy Products, 13th End., American Public Health Association, Inc., Washington, D.C.


# *Eosin Methylene Blue Agar (Levine)*

**LAB061**

**Description**

This medium was introduced in 1916 by Holt-Harris and Teague to differentiate *Escherichia* spp. and *Aerobacter* spp. It was modified by Levine in 1918 who removed sucrose from the formula and increased the lactose content. The distinctive metallic sheen produced by *E. coli* on this medium is due to acid production resulting in an amide bonding between the eosin and methylene blue, other coliforms do not produce enough acid to cause this reaction. Eosin inhibits most Gram positive organisms. The prepared medium is sensitive to light.

| Typical Formula | g/litre |
|---|---|
| Peptone | 10.0 |
| Lactose | 10.0 |
| Dipotassium phosphate | 2.0 |
| Eosin Y | 0.4 |
| Methylene Blue | 0.065 |
| Agar No. 2 | 15.0 |

**Method for reconstitution**

Weigh 37.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 50˚C and agitate gently to ensure uniform distribution of the flocculant precipitate (which is a feature of this medium) before pouring into Petri dishes.
STORE IN THE DARK.

**Appearance:** Blue/purple with a light precipitate.

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking for single colonies.

**Incubation:** 37˚C aerobically for 24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *E. coli* | 2.0-3.0 | CV.E.G. | Blue Black | (Metallic sheen) |
| *Klebsiella* spp. | 3.0-4.0 | CV.E.G. | Brown Blue | (mucoid) |
| *Salmonella* spp. | 2.0-3.0 | CV.E.G. | Colourless | |
| *S. aureus* | P.P. | CV.E.G. | Colourless | |
| *E. faecalis* | P.P. | CV.E.G. | Colourless | |

**References**


American Public Health Association, American Water Works Association and Water Pollution Control Federation, (1975). Standard Methods for the Examination of Water and Wastewater, 14th Edn., Washington, D.C. American Public Health Association.

Girolami, R.L. and Stamm, J.M. (1976). Inhibitory effect of light on growth supporting properties of Eosin Methylene Blue Agar. Appl. Environ. Microbiol., 31:1 141-142.

Haesler, W. J. (ed) (1972). Standard Methods for the Examination of Dairy Products, 13th edn., Washington, D.C., American Public Health Association.

Levine, M. (1918). Differentiation of *E. coli* and *B. aerogenes* on a simplified Eosin-Methylene Blue agar. J. Infect. Dis., 23: 43-47.

# *Eugon Agar*

***(Eugonic Agar)***

**LAB525**

**Description**

Eugon Agar is used for the cultivation of a wide variety of microorganisms, particularly in mass cultivation procedures. The medium is prepared according to the formulation of Vera and was developed to obtain eugonic (luxuriant) growth of fastidious microorganisms. The medium can be used with additions to enhance its performance with certain microorganisms, e.g. Eugon Agar supplemented with 5% sterile defibrinated blood will enable the growth of pathogenic fungi such as *Nocardia, Histoplasma* and *Blastomyces*.

Niven reported Eugon Agar for the detection of lactic acid bacteria in cured meats and recommended it for investigating spoilage in meats. Harrison and Hansen employed the medium for plate counts of the intestinal flora of turkeys and Frank showed its use for the germination of anaerobic spores pasteurised at 104°C. Eugon Agar is also specified in the APHA Compendium of Methods for the Microbiological Examination of Food.

The high sugar content of this medium dictates that it not suitable as a base for haemolytic reactions.

| Typical Formula | g/litre |
|---|---|
| Tryptose | 15.0 |
| Soy Peptone | 5.0 |
| Dextrose | 5.5 |
| L-Cystine | 0.7 |
| Sodium chloride | 4.0 |
| Sodium sulphite | 0.2 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 45.4 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C before the addition of supplements or pouring into sterile Petri dishes.

**Appearance:** Light amber clear gel, may contain a slight precipitate.

**pH:** 7.0 ± 0.2

Minimum QC organisms:
*Aspergillus niger* ATCC 16404
*Candida albicans* ATCC 10231
*Lactobacillus fermentum* ATCC 9388
*Streptococcus pyogenes* NCTC 8198

**Storage of Prepared Medium:** Plates can be stored up to 7 days at 2-8°C in the dark.

**Inoculation:** For the examination of clinical specimens for bacteria and fungi refer to the appropriate published references. For the examination of food for the examination of bacteria and fungi refer to standard methods.

**Incubation:** 35°C ± 2°C for up 72 ± 4 hours for bacteria. 30°C ± 2°C for up 72 ± 4 hours for fungi.

**Interpretation:** Refer to appropriate references and procedures.

**References**


Vera, H.D. (1947). The ability of peptones to support surface growth of lactobacilli. J. Bacteriol. 54:14.

MacFaddin, J.D. (1985). Media for the isolation-cultivation-identification-maintenance of medical bacteria. 301-303. vol. 1. Williams & Wilkens, MD.

Niven (1949). J. Bacteriol. 58:633.

Harrison, A.P.Jr. and Hansen, P.A. (1950). The bacterial flora of the cecal feces of healthy turkeys. J. Bacteriol. 59. 197.

Frank, H.A. (1955). The influence of various media on spore count determinations of a putrefactive anaerobe. J. Bacteriol. 70:269.

Vanderzant, C. and Splittstoesser, D.F. (ed.). (1992). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Isenberg, H.D. (ed.) (1992). Clinical microbiological procedures handbook, American Society for Microbiology, Washington, D.C.

Murray, P.R. *et al* (ed) (1995). Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Association of Official Analytical Chemists. (1995). Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.


# *Eugon Broth*

***(Eugonic Broth)***

**LAB526**

**Description**

This is the broth version of Eugon Agar (LAB525) for the cultivation of a wide variety of microorganisms, particularly in mass cultivation procedures. The medium is prepared according to the formulation of Vera and was developed to obtain eugonic (luxuriant) growth of fastidious microorganisms. The medium can be used with additions to enhance its performance with certain microorganisms e.g. Eugon Broth supplemented with 5% sterile defibrinated blood the medium will support the growth of pathogenic fungi such as *Nocardia, Histoplasma* and *Blastomyces*.

| Typical Formula | g/litre |
|---|---|
| Tryptose | 15.0 |
| Soy Peptone | 5.0 |
| Dextrose | 5.5 |
| L-Cystine | 0.7 |
| Sodium chloride | 4.0 |
| Sodium sulphite | 0.2 |

**Method for reconstitution**

Weigh 30.4 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool before the addition of enrichments and aseptically dispense into appropriate containers.

**Appearance:** Light amber solution, may contain a slight precipitate.

**pH:** 7.0 ± 0.2

Minimum QC organisms:
*Aspergillus niger* ATCC 16404
*Candida albicans* ATCC 10231
*Lactobacillus fermentum* ATCC 9388
*Streptococcus pyogenes* NCTC 8198

**Storage of Prepared Medium:** Store the prepared medium at 2-8°C.

**Inoculation:** For the examination of clinical specimens for bacteria and fungi refer to the appropriate published references.

**Incubation:** 35°C ± 2°C for up 72 ± 4 hours for bacteria. 30°C ± 2°C for up 72 ± 4 hours for fungi.

**Interpretation:** Refer to appropriate references and procedures.

**References**


Vera, H.D. (1947). The ability of peptones to support surface growth of lactobacilli. J. Bacteriol. 54:14.

MacFaddin, J.D. (1985). Media for the isolation-cultivation-identification-maintenance of medical bacteria. 301-303. vol. 1. Williams & Wilkens, MD.

Isenberg, H.D. (ed.) (1992). Clinical microbiological procedures handbook, American Society for Microbiology, Washington, D.C.

Murray, P.R. *et al* (ed) (1995). Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

# *Fastidious Anaerobe Agar (F.A.A.)*

**LAB090**

**Description**

A primary isolation medium capable of growing most clinically significant anaerobes. Developed by Lab M, comparisons have shown this medium to be superior to other formulations as a primary isolation medium for fastidious organisms. The peptones included have been chosen for maximum growth stimulation. Starch and sodium bicarbonate act as de-toxification agents whilst haemin encourages pigment production in *Porphyromonas melaninogenicus.* Specific growth promoting agents are Cysteine for *Fusobacterium necrophorum, Propionibacterium acne* and *Bacteroides fragilis,* arginine for *Eubacterium* spp. soluble pyrophosphate for *Porph. gingivalis* and *Porph. asaccharolytica.* Pyruvate helps neutralise hydrogen peroxide and is also utilised by *Veillionella* spp. as an energy source. Vitamin K and sodium succinate provide essential growth factors for some anaerobes as does the 0.1% glucose. The low level of glucose prevents the production of high levels of acids and alcohols which would inhibit colonial development.

| Typical Formula | g/litre |
|---|---|
| Peptone mix | 23.0 |
| Sodium chloride | 5.0 |
| Soluble starch | 1.0 |
| Agar No. 2 | 12.0 |
| Sodium bicarbonate | 0.4 |
| Glucose | 1.0 |
| Sodium pyruvate | 1.0 |
| Cysteine HCl monohydrate | 0.5 |
| Haemin | 0.01 |
| Vitamin K | 0.001 |
| L-Arginine | 1.0 |
| Soluble pyrophosphate | 0.25 |
| Sodium succinate | 0.5 |

**Method for reconstitution**

Weigh 46 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C then aseptically add 5-10% of sterile defibrinated horse blood, mix well and pour into Petri dishes. This medium can be made selective for various species of anaerobes by the addition of appropriate selective cocktails e.g.

| | |
|---|---|
| Gram negative anaerobes | X090 |
| Non-sporing anaerobes | X291 |
| *Clostridium difficile* | X093 |

**Appearance:** Red due to addition of blood. The blood will darken (reduce) because of the presence of reducing agents.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *B. fragilis* ATCC 25285
*P. anaerobious* ATCC 27337

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface plating, streaking out to single colonies.

**Incubation:** 37˚C anaerobically with 10% $CO_2$ for 48 hours to 5 days.

**Growth Characteristics (48 hours)**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Bacteroides fragilis* | 1.0 - 2.0 | CV.E.G. | Grey | |
| *Clostridium Perfringens* | 1.0 - 2.0 | CV.E.G | Grey | 'Target' haemolysis (non haemolytic) |
| *Fusobacterium necrophorum* | 1.0 - 2.0 | CV.E.G (D) | trans-parent | (grey) (haemolytic) |
| *Porphyromonas asaccharolyticus* | 1.0 - 2.0 | CV.E.G | Grey / Brown | (clearing) |
| *Peptostreptococcus anaerobius* | 0.5 - 2.0 | CV.E.G | White / Grey | |
| *Actinomyces israeli* | 0.5 - 1.0 | CV.E.G | White | ('molar tooth') (smooth) |

**References**


Brazier, J.S. (1986). Yellow fluorescence of Fusobacteria Letters in Applied Microbiol. 2: 124-126.

Brazier, J.S. (1986). A note on ultra violet red fluorescence of anaerobic bacteria in vitro. J. Appl. Bact. 60: 121-126.

Eley, A., Clarry, T., Bennett, K.W. (1989). Selective and differential medium for isolation of *Bacteriodes ureolyticus* from clinical specimens. European Journal of Clinical Microbiology, Infectious Diseases. 8: 83-85.

Wade W. Griffiths, M. (1987). Comparison of Media for cultivation of subgingival bacteria. J. Dent. Res. 66: no. 4 abstract 334.

Heginbotham M., Fitzgerald T.C., and Wade W.G. (1990). Comparison of solid media for the culture of anaerobes. J. Clin. Path. 43: 253-256.


# *Fastidious Anaerobe Broth (F.A.B.)*

**LAB071**

**Description**

F.A.B. was developed by Lab M working in conjunction with the microbiology department of a University of Manchester teaching hospital. The medium was designed to give optimum growth of fastidious anaerobes and has found applications as a blood culture medium and an enrichment broth for the isolation of anaerobes. The medium is very rich in nutrients from the specially selected peptone mixture. Vitamin K. haemin and L-cysteine are all growth factors required by some anaerobes. L-cysteine together with sodium thioglycollate reduce the Eh of the medium and the agar content inhibits absorption of oxygen and convection currents. Resazurin is a redox indicator. Several published evaluations show F.A.B. to be the liquid medium of choice for fastidious anaerobes.

| Typical Formula | g/litre |
|---|---|
| Peptone mixture | 15.0 |
| Yeast Extract | 10.0 |
| Sodium thioglycollate | 0.5 |
| Sodium chloride | 2.5 |
| Agar No. 1 | 0.75 |
| L-Cysteine HCl | 0.5 |
| Resazurin | 0.001 |
| Sodium bicarbonate | 0.4 |
| Haemin | 0.005 |
| Vitamin K | 0.0005 |

**Method for reconstitution**

Weigh 29.7 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix. Boil to dissolve the agar then dispense into screw cap containers. Sterilise by autoclaving at 121˚C for 15 minutes. Tighten the caps as soon as possible after autoclaving.

**Appearance:** Pale straw, clear, viscous. May have a narrow band of red/purple at the surface due to action of oxygen on the resazurin. If the medium is reddish this indicates too much oxygen has been absorbed, the medium should be reheated to deoxygenate. Do not reheat more than once.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *Bacteroides fragilis* ATCC 25285

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** If used as a blood culture medium a minimum dilution of 1:10 should be used.

**Incubation:** 37˚C for 24-72 hours. Keep the container airtight.

**Growth indicators:** The broth may become turbid or individual colonies may form suspended in the medium.

**References**


Gould, J.H., Duerden, B.I. (1983). Blood culture – current state and future prospects. J. Clin. Pathol. 36: 963-977.

Ganguli, G.A., O'Hare, W., Hyde, W.A. (1984). Rapid Detection of Bacteraemia by early subculture. J. Med. Microbiol. 17: 311-315.

Ganguli, L.A., Keaney, M.G.L., Hyde, W.A., Fraser, B.J. (1985). More Rapid identification of bacteraemia by manual rather than radiometric methods. J. Clin. Pathol. 38: 1146-1149.

Junt, G.H., Price, E.H. (1982). Comparison of a home made blood culture broth containing a papain digest of liver, with four commercially available media, for the isolation of anaerobes from simulated paediatoic blood cultures. J. Clin. Pathol. 35: 1142-1149.

Ganguli, L.A., Turton, L.J., Tillotson, G.S. (1982). Evaluation of Fastidious Anaerobe Broth as a blood culture medium. J. Clin. Pathol. 35: 458-461.

Tillotson, G.S. (1981). Evaluation of ten commercial blood culture systems to isolate a pryridoxal dependent streptococcus. J. Clin. Pathol. 34: 930-934.


# *Fluid Thioglycollate Medium (Clear)*

**LAB425**

**Description**

A medium for sterility tests to promote growth in both Aerobic and anaerobic organisms even from small inocula. In appropriate tubes or bottles the thioglycollate ensures adequate anaerobic conditions. The low level of agar reduces oxygen diffusion into the medium.

Performance of this medium complies with the requirements described in the EP/USP/JP for Fluid Thioglycollate Medium.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 15.0 |
| L-Cystine | 0.5 |
| Glucose | 5.5 |
| Yeast Extract | 5.0 |
| Sodium chloride | 2.5 |
| Sodium thioglycollate | 0.5 |
| Resazurin | 0.001 |
| Gelling agent | 0.75 |
| **Grams per litre** | **29.75** |

**Method for reconstitution**

Weigh 29.75 grams, disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix, then bring to the boil to dissolve and dispense into suitable airtight containers. Sterilise by autoclaving for 15 minutes at 121°C. Store the medium between 2°C - 25°C in the dark.

If more than 30% of the medium turns pink (oxidised) the Eh may be restored (once only) by heating in a boiling water bath or by free-flowing steam. Take care to cool quickly after heating and prevent the introduction of non-sterile air into the container.

**Appearance:**

Powder: fine, free - flowing, homogeneous, buff

Finished medium: Pale straw colour, clear. Surface may be pink due to oxidation of Resazurin

**pH:** 7.1 ± 0.2

**Hazard classification**
NR – Not regulated

Minimum Q.C. organisms:
*Clostridium sporogenes* ATCC 19404
*Pseudomonas aeruginosa* ATCC 9027
*Staphylococcus aureus* ATCC 6538

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: Capped container – up to 3 months at 15-20°C in the dark.

**Incubation:** 30-35°C aerobically for 14 days.

**Interpretation:** Turbidity, colonies in medium.

# *Fluid Thioglycollate Medium (USP/EP/JP)*

**HP001**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for sterility testing. Conforms to USP/EP/JP performance specification. Casein and yeast extract provide a source of nitrogen, essential vitamins and amino acids. The glucose provides a carbon source and sodium chloride maintains osmotic balance. L-Cystine and sodium thioglycollate act as reducing agents to create an anaerobic environment and maintain a low Eh. This is aided by the low level of agar which reduces the oxygen permeability through the medium. Resazurin is an oxidation indicator which turns from colourless to red/pink when oxidised. Sodium thioglycollate also serves to inactivate mercurial compounds. If after sterilisation more than the upper one third of the medium has become red/pink it may be restored once by heating in a water bath or in free-flowing steam until the colour disappears. Ensure the media is cooled quickly and prevent the introduction of non-sterile air into the containers.

| Typical Formula | g/litre |
|---|---|
| L-Cystine | 0.5 |
| Agar | 0.75 |
| Sodium chloride | 2.5 |
| Glucose anhydrous | 5.0 |
| Yeast extract | 2.5 |
| Pancreatic digest of casein | 15.0 |
| Sodium thioglycollate | 0.3 |
| Resazurin | 0.001 |

**Method for reconstitution**

Disperse 26.55 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and bring to the boil. Distribute into suitable vessels and sterilise at 121°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw, clear to slight haze with red/pink layer at the top of the medium

**pH:** 7.1 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* ATCC 6538
*Bacillus subtilis* ATCC 6633
*Pseudomonas aeruginosa* ATCC 9027
*Candida albicans* ATCC 10231
*Clostridium sporogenes* ATCC 19404
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Use:** According to the sterility protocol defined in the Harmonised European Pharmacopoeia the samples are incubated in portions of the medium at 30-35°C for 14 days. No growth of micro-organisms is required for a sterility pass.

According to the growth promotion test defined in the Harmonised European Pharmacopeia, *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus* are inoculated (with not more than 100 CFU) and incubated for not more than 3 days. The media is suitable if a clearly visible growth of the micro-organisms occurs.

**Interpretation:**

Growth is indicated by turbidity, refer to specific guidelines as defined in the Harmonised European Pharmacopoeia.

**References**

European Pharmacopoeia 8th Edition

# *Fraser Broth*

**LAB164**

**Description**

Developed as a modification of UVM II medium, Fraser broth is a secondary enrichment broth for the isolation of *Listeria* spp., and is similar to Palcam broth in that it contains aesculin to indicate the presence of a potential *Listeria* isolate. It also contains lithium chloride in an attempt to suppress the growth of enterococci in the medium (as does Palcam). Fraser broth may also be used as a primary enrichment medium by incorporating 1/2 strength supplement into the broth base (X164 or X564).

| Typical Formula | g/litre |
|---|---|
| Peptone mixture | 15.0 |
| Yeast extract | 5.0 |
| Aesculin | 1.0 |
| Disodium hydrogen phosphate | 9.6 |
| Potassium dihydrogen phosphate | 1.35 |
| Sodium chloride | 20.0 |
| Lithium chloride | 3.0 |

**Method for reconstitution**

Weigh 55 grams of power and add to 1 litre of deionised water (add to 900ml if preparing 1/2 Fraser). Allow to soak for 10 minutes, swirl to mix and sterilise at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of Fraser supplement X165 (or 2 vials of 1/2 Fraser supplement X164), mix well and aseptically dispense into sterile tubes or bottles.

**Appearance:** Straw opalescent broth with precipitate (clears on storage).

**pH** 7.2 ± 0.2

Minimum Q.C. organisms:
*Listeria monocytogenes* WDCM 00021
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Bottles – up to 14 days at 2-8˚C.

**Inoculation:** 1/2 Fraser – Add 25g sample to 225ml of 1/2 Fraser broth and homogenise Fraser – Subculture 0.1ml of primary enrichment broth (UVM I or 1/2 Fraser) into 10ml of Fraser broth.

**Incubation:** 1/2 Fraser – 30˚C aerobically for 24hrs.

Fraser – 35˚C aerobically for 24hrs and 48hrs. Subculture onto selective agars at 24 and 48hrs.

**Interpretation**

Blackening of the broth indicates the presence of a potential Listeria and should be subcultured onto Listeria isolation medium (Oxford) LAB122 or Palcam agar LAB148. All broths should be subcultured before discarding, irrespective of colour change.

**References**

Fraser J.A., and Sperber W.H., (1988) Rapid detection of Listeria spp in food and environmental samples by esculin hydrolysis. J.Food Protection 51 (10) 762-765.

McClain D., and Lee W.H. (1989) FSIS method for isolation of L.monocytogenes from processed meat and poultry products. Lab.Comm.No.57, Revised May 24, (1989). US Dept of Agric.FSIS, Microbiol. Div.

# *Fraser Broth$^{PLUS}$ (ISO)*

**LAB212**

**Description**

A secondary enrichment broth for the isolation of *Listeria* spp. formulated according to ISO 11290. The selective components acriflavine and nalidixic acid are blended into the base powder and the ferric ammonium citrate (X211) is added to the tempered broth after sterilisation.

| Typical Formula | g/litre |
|---|---|
| Peptone mixture | 15.00 |
| Yeast extract | 5.00 |
| Aesculin | 1.00 |
| Disodium hydrogen phosphate | 9.60 |
| Potassium dihydrogen phosphate | 1.35 |
| Sodium chloride | 20.00 |
| Lithium chloride | 3.00 |
| Acriflavine | 0.025 |
| Nalidixic acid | 0.02 |

**Method for reconstitution**

Weigh 55 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and add 2 vials X211. Mix well and dispense into sterile containers..

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw opalescent broth with yellow fluorescence (in final medium).

**pH:** 7.2 ± 0.2

**Hazard classification**
NR – Not regulated

Minimum Q.C. organisms:
*Listeria monocytogenes* WDCM 00021
*Escherichia coli* WDCM 00013
*Enterococcus faecalis* WDCM 00087

**Storage:**

Dehydrated culture media: 10-25°C

Final medium: 14 days at 2-8°C in the dark

**Inoculation:** Sub-culture 0.1mL of LAB211 into 10mL of LAB212 Fraser Broth$^{PLUS}$ (ISO).

**Incubation:** 35-37°C aerobically for 24hrs and 48hrs. Sub-culture onto selective agar at 24 and 48 hours.

**Interpretation:** Blackening of the broth indicates the presence of a potential *Listeria* spp. and should be sub-cultured onto a selective *Listeria* isolation medium, e.g. Harlequin™ Listeria Chromogenic Agar (HAL010). All broths should be sub-cultured before discarding irrespective of colour change.

**References**

Fraser J.A. and Sperber W.H. (1988). Rapid detection of Listeria spp in food and environmental samples by esculin hydrolysis. *J. Food Protect.* 51, No.10, 762-765.

McClain D. and Lee W.H. (1989). FSIS method for isolation of L. monocytogenes from processed meat and poultry products. Lab. Comm.No.57, Revised May 24, (1989). US Dept of Agric.FSIS, Microbiol. Div.

ISO 11290-1:1997 (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Listeria monocytogenes* - part 1, Incorporating Amendment 1.)

# *G.C. Agar Base*

**LAB067**

**Description**

A nutritious agar base described by Thayer and Martin for the isolation of Neisseria gonorrhoeae. The rich peptone mixture is enhanced by the use of corn starch to absorb toxic metabolites and a buffering system is used to maintain neutral pH. The medium is made selective by the use of various antibiotic cocktails. Thayer and Martin originally recommended the use of vancomycin, colistin and nystatin V.C.N. but the addition of trimethoprim (X068) is useful in preventing the swarming of proteus. More recently the emergence of vancomycin sensitive gonococci has made the New York City selective agents (lincomycin, colistin, amphotericin, trimethoprim X070, LCAT) the combination of choice. Enrichment of the base is usually by the addition of lysed blood. Alternatively chocolated blood or haemoglobin powder and Thayer and Martin's mixture of vitamins, amino acids and coenzymes can be used. The growth supplement X271 can be added to this medium to aid in the isolation of *Neisseria* spp.

| Typical Formula | g/litre |
|---|---|
| Special Peptone | 15.0 |
| Corn Starch | 1.0 |
| Sodium chloride | 5.0 |
| Dipotassium hydrogen phosphate | 4.0 |
| Potassium dihydrogen phosphate | 1.0 |
| Agar No. 2 | 10.0 |

**Method for reconstitution**

Weigh 36 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Cool to 48°C and add 50-70ml of lysed blood and 2 vials of X070 selective agent. Mix well and pour into Petri dishes.

**Appearance:** Dependent on blood supplement used.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *N. gonorrhoeae* NCTC 8375
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C in the dark.

**Inoculation:** Surface, streaking out for single colonies.

**Incubation:** 37°C microaerobically for 24-48 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *N. gonorrhoeae* | 1.0-2.0 | CV.E.G. | Transparent | variations in colony size |

**References**

Young, H. 1978. Cultural diagnoses of gonorrhoea with modified New York City (MNYC) medium. Brit. Journ. Ven. Dis. 54: 36-40:

Thayer, J. D. and Martin, J. E. 1966. Improved medium selective for the cultivation of *N. gonorrhoeae* and *N. Meningitidis:* Public Health rep. 81: 559-562.

# *GVPC Legionella Isolation Medium (BCYE basal medium)*

**LAB195**

**Description**

BCYE (Buffered Charcoal Yeast Extract) Legionella Isolation Medium (LAB195) is a base medium used for the isolation of Legionella from clinical and environmental samples. This medium is based on the charcoal yeast extract formulation of Feeley *et al*.[1&2] The performance of this medium is further enhanced by the additions of ACES (N-2-acetamido-2-aminoethane - sulphonic acid) buffer and α-ketoglutarate as defined by Edelstein[3]. This medium is also detailed in internationally recognized methodology[4] for the isolation of *Legionella* spp. from water.

Specimens or samples are often heavily contaminated with other bacteria and consequentially a range of selective supplements have been developed to aid isolation. Lab M provide the GVPC supplement (X195) which is most effective for the isolation of *L. pneumophila*. It is recommended that this supplement is used in conjunction with heat and acid sample treatments, to further reduce the growth of non-*Legionella* bacteria.

This product contains the ACES buffer and ferric pyrophosphate in the base medium. This negates the need for complex freeze dried supplements. A complementary growth supplement is provided (X196) which contains the L-cysteine and α-ketoglutarate.

**Principle of isolation**

Water samples are concentrated either by membrane filtration or centrifugation (turbid samples may also be centrifuged). To reduce the growth of unwanted bacteria, separate portions of the concentrated sample may be subjected to heat and acid treatments. Treated and untreated portions are then inoculated onto *Legionella* selective media.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 10.0 |
| Charcoal | 2.0 |
| Ferric | 0.25 |
| Pyrophosphate ACES Buffer | 10.0 |
| Potassium Carbonate | 2.28 |
| Agar | 14.0 |

**Supplements**

| GVPC Selective Supplement (X195) | |
|---|---|
| **Typical Formula** | |
| Glycine | 3000mg |
| Vancomycin | 1mg |
| Polymyxin B | 79200IU |
| Cycloheximide | 80mg |

| BCYE Growth Supplement (X196) | |
|---|---|
| **Typical Formula** | |
| L-Cysteine | 400mg |
| α-ketoglutarate | 1000mg |

**Method for reconstitution**

**Selective Isolation (GVPC BCYE)**

Weigh 38.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and sterilise by autoclaving at 110ºC for 10 minutes. Cool to 47ºC and aseptically add 2 vials of reconstituted growth supplement X196 and 2 vials of reconstituted selective supplement X195. Mix well and pour into sterile Petri dishes.

**pH:** 6.9 ± 0.1

**Inoculation:**

The concentrated sample should be split into 3 portions. One portion is used without any further treatment, the other 2 portions should be treated, one with heat and the other with acid.

Heat Treatment

Take 1ml of the concentrated sample and place in a water bath at 50ºC for 30 minutes.

Acid Treatment

Take 1-10ml of the concentrated sample and centrifuge at 6000g for 10 minutes. Decant the supernatant to leave half the original volume. Vortex to re-suspend the pellet and make up to the original volume using an HCl-KCl buffer. Leave to stand for 5 minutes.

Inoculate the first plate of GVPC supplemented media with 0.1mL of the untreated portion and spread over the entire surface of the plate. Inoculate the second plate of GVPC supplemented media in the same way with 0.1ml of the heat treated portion as soon as possible after removal from the water bath. Inoculate the third plate of GVPC supplemented media in the same way with 0.1mL of the acid treated portion immediately after acid treatment.

**Incubation:**

Incubate at 36 + 1ºC in a humid atmosphere under aerobic conditions for up to 10 days.

**Interpretation:**

The plates should be examined for growth on days 3, 5, 7 and 10. Suspect colonies should be sub-cultured on to "maintenance" supplemented BCYE medium and "presumptive ID" supplemented BCYE medium, incubate as before. Isolates that fail to grow on the "presumptive ID" medium but grow on the maintenance medium and have typical morphology should be regarded as presumptive *Legionella*.

Presumptive isolates should be confirmed using a serological method, e.g. Microgen M45 Latex.

Minimum Q.C. organisms: *Legionella* spp. - Growth
*Staphylococcus epidermidis* - Growth
*Escherichia coli* - Inhibited

**References**


Feeley, J.C., Gibson, R.J. *et al.* (1979). *Journal of Clinical Microbiology* **10**: 437-441

Pesculle, A.H., Feeley, J.C. *et al.* (1980). *Journal of Infectious Disease* **141**: 727-732

Edelstein, P.H. (1982). *Journal of Clinical Microbiology* **14**: 298-303

International Standard. ISO 11731:1998(E). Water Quality – Detection & Enumeration of *Legionella*.


# *Half Fraser Broth$^{PLUS}$ (ISO)*

**LAB211**

**Description**

Primary enrichment broth for the isolation of *Listeria* spp. formulated according to ISO 11290. The selective components acriflavine and nalidixic acid are blended into the base powder and the ferric ammonium citrate (X211) is added to the tempered broth after sterilisation.

Half Fraser Broth Plus (ISO), LAB211 was developed to give improved results for the isolation of *Listeria* spp. with both traditional and ELISA methods. As with the sister product, Fraser Broth Plus (ISO), LAB212 the selective components acriflavine and nalidixic acid are blended into the base powder and the Ferric Ammonium Citrate (X211) is added to the tempered broth after autoclaving. This format has been shown to give improved selectivity with pure cultures and food samples. Furthermore, more stable ELISA results are seen, resulting in fewer false positive results when the ferric ammonium citrate is omitted from the complete media.

Developed as a modification of UVM medium and made according to ISO 11290, Fraser Broth is a secondary enrichment broth for the isolation of *Listeria* spp., and is similar to Palcam Broth in that it contains aesculin to indicate the presence of a potential *Listeria* isolate.

It also contains lithium chloride in an attempt to suppress the growth of Enterococci in the medium (as does Palcam). *Listeria* spp. hydrolyse the aesculin to form aesculetin, which reacts with the ferric ammonium citrate in X211 resulting in a black precipitate and a visible positive reaction. However, Enterococci can also perform this reaction, so further plating is required onto an isolation medium such as Harlequin™ Listeria Chromogenic Agar ISO (HAL010).

Note: Acriflavine and Nalidixic Acid in LAB211 are half-strength of Fraser Broth$^{PLUS}$ (ISO) LAB212.

| Typical Formula | g/litre |
|---|---|
| Peptone mixture | 15.00 |
| Yeast extract | 5.00 |
| Aesculin | 1.00 |
| Disodium hydrogen phosphate | 9.60 |
| Potassium dihydrogen phosphate | 1.35 |
| Sodium chloride | 20.00 |
| Lithium chloride | 3.00 |
| Acriflavine | 0.0125 |
| Nalidixic acid | 0.01 |

**Method for reconstitution**

Weigh 55 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and add 2 vials X211. Mix well and dispense into sterile containers.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: straw opalescent broth with yellow fluorescence (in final medium).

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Listeria monocytogenes* WDCM 00021
*Escherichia coli* WDCM 00013
*Enterococcus faecalis* WDCM 00087

**Storage:**

Dehydrated culture media: 10-25°C
Final medium: 14 days at 2-8°C in the dark

**Inoculation:** Add 25g of sample to 225mL LAB211 and homogenise. Sub-culture 0.1mL of LAB211 into 10mL of LAB212 Fraser Broth$^{PLUS}$ (ISO).

**Incubation:** 30°C aerobically for 24 hours.

**Interpretation:** Blackening of the broth indicates the presence of a potential *Listeria* spp. All broths should be sub-cultured before discarding irrespective of colour change.

**References**


Fraser J.A. and Sperber W.H. (1988). Rapid detection of Listeria spp in food and environmental samples by esculin hydrolysis. *J. Food Protect.* 51, No.10, 762-765.

McClain D. and Lee W.H. (1989). FSIS method for isolation of L. monocytogenes from processed meat and poultry products. Lab. Comm.No.57, Revised May 24, (1989). US Dept of Agric.FSIS, Microbiol. Div.

ISO 11290-1:1997 (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Listeria monocytogenes* - part 1, Incorporating Amendment 1.)


## *Hektoen Enteric Agar*

**LAB110**

**Description**

A medium developed at the Hektoen Institute in Chicago for the enhanced recovery of shigellae from clinical specimens. This medium has high levels of peptones and sugar which counteract some of the toxic effects of bile salts used to make the medium selective. This allows the shigellae to grow as well as the salmonellae. Salicin is fermented by many coliforms including those that do not ferment lactose and sucrose. The medium employs a double indicator system similar to that used in LAB006 C.L.E.D., (Bevis) and an $H_2S$ indicator system similar to that used in LAB032 XLD. Although intended primarily for clinical use this medium is quoted in B.S. 4285 as suitable for the examination of dairy products for salmonellae.

| Typical Formula | g/litre |
|---|---|
| Meat Peptone | 12.0 |
| Yeast Extract | 3.0 |
| Lactose | 12.0 |
| Sucrose | 12.0 |
| Salicin | 2.0 |
| Bile Salts No. 3 | 7.0 |
| Sodium desoxycholate | 2.4 |
| Sodium chloride | 5.0 |
| Sodium thiosulphate | 5.0 |
| Ammonium ferric citrate | 1.5 |
| Acid fuchsin | 0.1 |
| Bromothymol blue | 0.065 |
| Agar No. 1 | 14.0 |

**Method for reconstitution**

Weigh 76 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then heat gently and bring to the boil. Cool to 47˚C and pour plates. DO NOT AUTOCLAVE OR OVERHEAT THIS MEDIUM.

**Appearance:** Green, clear.

**pH:** 7.5 ± 0.2

Minimum Q.C. organisms:
*Salmonella typhimurium* WDCM 00031
*Shigella* sp.
*E. coli* (some inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface plating, streak out to single colonies.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| $H_2S$ +ve *Salmonella* | 2-3 Black | CV.E.G. | Green+ | |
| $H_2S$ -ve *Salmonella* | 2-3 | CV.E.G. | Green | |
| *S. sonnei* | 2-2.5 | CV.E.G. | Green | (Rough) |
| *E. coli* | 0.5-2 | CV.E.G. | Salmon ppt. around colonies | (Rough) (No growth) |
| *Citrobacter* spp. | 1.0-2.0 | CV.E.G. | Salmon | (Rough) |
| *Proteus* spp. | 1.0-2.0 | CV.E.G. | Green/ Black centre | (No growth) (brownish centre) |

**References**


King, S. and Metzger, W.I. (1967). A new medium for the isolation of *Salmonella* and *Shigella* species. Bact. Proc. Am. Soc. Microbiol. 77.

King, S. and Metzger, W.I. (1968). A new plating medium for the isolation of enteric pathogens. Hektoen Enteric Agar, Appl. Microbiol., 16(4), 577.

King, S. and Metzger, W.I. (1968). A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Agar with SS and EMB agar. Appl. Microbiol., 16(4), 579.

Speck, M.L. (ed.). (1976). Compendium of Methods for the Microbiological Examination of Food. Washington, D.C.: American Public Health Association.


## *Hoyle's Medium (modified)*

**LAB027**

**Description**

A highly selective culture medium for the isolation and differentiation of *Corynebacterium diphtheriae* types gravis, mitis and intermedius. This product, based on Hoyle's medium gives rapid growth of all types of *C. diphtheriae,* which results in most specimens giving adequate growth with overnight incubation.

| Typical Formula | g/litre |
|---|---|
| Peptone | 1.0 |
| Yeast Extract | 4.0 |
| Sodium chloride | 4.0 |
| Sugars | 1.0 |
| Agar | 12.0 |

**Method of Reconstitution**
Weigh 37 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C, add 50ml of lysed horse or sheep blood and 10ml of X027 potassium tellurite solution. Mix well before pouring.

**Appearance:** Dark Red, clear gel

**pH:** 7.8 ± 0.2

**Inoculation:** Spread the entire surface with the swab or sample under investigation. Hoyle's medium is very selective and spreading for single colonies using a wire loop is not necessary. Use of a non-selective blood agar alongside Hoyle's is recommended.

**Incubation:** 37˚C for 18-48 hrs, aerobically

**Storage:** Plates – up to 7 days at 2-8˚C

**Interpretation**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *C. diphtheriae var mitis* | 0.5-2.0 | CV.E.G. | Grey (dark centre) | Easily emulsified |
| *Streptococcus* Spp. | pp-1.5 | CV.E.G | Black | Enterococci may be larger |

Minimum Q.C. Organisms *C. diphtheriae var mitis* (non-toxigenic) NCTC 13056
*E. coli* WDCM 00013 (inhibition)

**Reference:**
Hoyle L. (1941) A Tellurite Blood Agar Medium for the Rapid Diagnosis of Diphtheria. Lancet 1 175-176

176. Elek S.D. (1948) The Recognition of Toxigenic Bacterial Strains in vitro. Brit. Med. J. 1 493-496.

## *Iron Sulphite Agar*

**LAB222**

**Description**
Iron Sulphite Agar is a medium for the detection of thermophilic anaerobic organisms causing sulphide spoilage in food.

This formulation is a modification of Cameron Sulphite Agar, which was developed by the National Canners Association of America (now the Grocery Manufacturers Association).

Iron Sulphite Agar has a reduced concentration of sodium sulphite to allow improved detection of some strains of *Clostridium sporogenes*. Beerens, and later Mossel, demonstrated that some strains of *C. sporogenes* would not tolerate sodium sulphite levels of 0.1%. Mossel further observed that reducing sulphite content to 0.05% improved detection of these strains.

Tryptone provides nitrogen and other nutrients necessary to support bacterial growth. The presence of sulphite reducing bacteria is indicated by the formation of black colonies. These colonies form when bacteria reduce sulphite to sulphide, which reacts with iron (III) citrate to yield a black precipitate.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Sodium sulphite | 0.5 |
| Iron (III) citrate | 0.5 |
| Agar | 12.0 |
| **Grams per litre** | **23.0** |

**Appearance:**
Powder: fine, free-flowing, homogeneous, buff with brown flecks
Finished medium: clear, light tan gel

**pH:** 7.1 ± 0.2

**Hazard classification**
NR – Not regulated

**Method for reconstitution**
Disperse 23 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and mix well before dispensing.

**Storage**
Dehydrated culture media: 10-25°C.
Final medium: Use on day of preparation

**Inoculation**
Use 'deep shake' or 'Attenborough and Scarr overlay' methods for inoculation.

Deep-Shake Culture Method
Dispense the medium in 10ml volumes in tubes. Inoculate the sample when the medium is at approximately 50°C. Allow to set.

Attenborough and Scarr Method
This membrane filter technique is quicker, of comparable accuracy and permits the examination of larger samples.
In this method, diluted samples of sugar or any other food are filtered through membrane filters. These filters are then rolled up and placed in tubes containing just sufficient Iron Sulphite Agar (at 50°C) to cover them. The medium is allowed to set.

**Incubation**
Incubate for 24-48 hours at 55°C for thermophilic organisms or 37°C for mesophilic organisms. May also be used for mesophillic sulphite reducers if incubated at 37°C.

**Interpretation**
Deep-Shake Culture Method
Typical thermophilic species, e.g. *Desulfotomaculum nigrificans* , produce distinct black spherical colonies in the depth of the medium.

Attenborough and Scarr Method
Count the number of black colonies on the membrane filter. Confirmation tests should be carried out to identify the organism growing in the medium.

The blackening reaction is only presumptive evidence of clostridial growth. Confirmation tests must be carried out for identification. There are many gram-negative bacteria that are able to reduce sulfite with iron sulfide production in this medium, but in these cases the enzymes are extra cellular and the entire medium becomes dark, rendering their enumeration impossible.

Minimum Q.C. organisms:
*Desulfotomaculum nigrificans* ATCC 7946
*E.coli* WDCM 00013
*C.sporogenes* WDCM 00008

**References**
Attenborough, S.J. & Scarr, P.M. (1957). *J. Appl.* Bact. **20**. p460-466.

Beerens, H. (1958) DSIR. Proc. 2nd Internat. Symp. Food. Microbiol. 1957, HMSO, London, pp. 235-245.

Bufton, A.W.J. (1959). *J. Appl.* Bact. **22**. p278-280.

Mossel, D.A.A., Golstein Brouwers, G.W.M.V. & de Bruin, A.S. (1959). J. Path. Bact. **78**. 290-291.

Tanner, F.W. (1944). The Microbiology of Foods, 2nd edition, Garrard press, Illinois, p1127.

## *Kanamycin Aesculin Azide Agar*

***(K.A.A. Agar)***

**LAB106**

**Description**
A selective isolation and enumeration medium for enterococci (Lancefield group D streptococci) in food. Sodium azide and kanamycin provide the selective inhibition required whilst aesculin and iron salts form an indicator system for the presumptive identification of enterococci. Incubation at 42˚C will increase the medium's selectivity.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 5.0 |
| Sodium citrate | 1.0 |
| Aesculin | 1.0 |
| Ferric ammonium citrate | 0.5 |
| Sodium azide | 0.15 |
| Kanamycin sulphate | 0.02 |
| Agar No. 1 | 10.0 |

**Method for reconstitution**

Weigh 43 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C, then dispense into Petri dishes.

**Appearance:** Pale straw, clear.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *E. faecalis* WDCM 00087
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, spread 0.1ml to 0.5ml over entire surface of plate.

**Incubation:** 37˚C or 42˚C aerobically for 18-24 hours.

**Interpretation:** Count all white/grey colonies, approx 2mm diameter, surrounded by a black halo to give presumptive enterococcus/faecal streptococcus count.

**References**

Mossel, D.A.A., Bijken, P.H.G., Eelderink, I. and van Spreekens, K.A. (1978). Streptococci, edited by Skinner, F. A. and Quesnel, L.B. SAB Symposium Series No. 7 Academic Press, London.

## *Kanamycin Aesculin Azide Broth*

***(K.A.A. Broth)***

**LAB107**

**Description**

An enrichment and isolation medium for enterococci. The medium can be used with the M.P.N. technique to enumerate enterococci in food. This broth is identical to LAB106 K.A.A. agar with the omission of the agar.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 5.0 |
| Sodium citrate | 1.0 |
| Aesculin | 1.0 |
| Ferric ammonium citrate | 0.5 |
| Sodium azide | 0.15 |
| Kanamycin sulphate | 0.02 |

**Method for reconstitution**

Weigh 33 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, warm gently to dissolve completely then disperse into tubes or bottles. Sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Light straw, clear.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *E. faecalis* WDCM 00087
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Inoculate tubes with decimal dilutions of food suspension.

**Incubation:** 37˚C or 42˚C aerobically for 18-24 hours.

**Interpretation:** Blackening of the medium suggests the presence of enterococci/faecal streptococci.

**References**

Mossel, D.A.A., Bijken, P.H.G., Eelderink, I. and. van Spreekens. K.A. (1978). Streptococci, edited by Skinner, F.A. and Quesnel, L.B. SAB Symposium Series No. 7 Academic Press, London.

## *Kligler Iron Agar*

**LAB059**

**Description**

A differential medium for the recognition of enteric pathogens by their ability to ferment glucose and/or lactose, and liberate sulphides. Fermentation liberates acid, with or without gas, turning phenol red indicator yellow. Fermentation of glucose only, is followed by reversion in pH on the slope, from initial acidity to final alkalinity (red colour), but not in the anaerobic conditions of the butt, which remains acid (yellow). Fermentation of lactose as well as glucose, produces acidity in both slope and butt (yellow). Liberation of sulphide results in the formation of iron sulphide (blackening of either slope or butt).

| Typical Formula | g/litre |
|---|---|
| Peptone | 20.0 |
| Lactose | 10.0 |
| Glucose | 1.0 |
| Sodium chloride | 5.0 |
| Ferric ammonium citrate | 0.5 |
| Sodium thiosulphate | 0.3 |
| Phenol red | 0.025 |
| Agar No. 2 | 12.0 |

**Method of reconstitution**

Weigh 49 grams of powder and mix with 1 litre of distilled water. Bring to the boil with frequent stirring to dissolve completely. Dispense into tubes and sterilise for 15 minutes at 121˚C. Cool in a slanted position such that slopes are formed over deep butts approx. 3cm in depth.

**Appearance:** Reddish brown agar.

**pH:** 7.4 ± 0.2

Minimum Q.C. Organisms *Salmonella typhimurium* WDCM 00031
*Pseudomonas aeruginosa* WDCM 00025

**Inoculation**

Subcultures for further identification are picked from the centre of isolated colonies on selective media and streaked across the slant and stabbed deep into the butt of tubes of Kligler Iron Agar.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Interpretation**

| Organism | Butt | Slope | Sulphide |
|---|---|---|---|
| *Salmonella typhi* | Acid | Alkaline | + |
| *S. paratyhi* A+ B | Acid | Alkaline | - |
| Other *Salmonella* | Acid/gas | Alkaline | + |
| *E. coli* | Acid/gas | Acid | - |
| *Proteus* spp | Acid/gas | Alkaline | + |
| *Shigella sonnei* | Acid | Alkaline | - |
| *S. flexneri* | Acid | Alkaline | - |

**Storage:** Tightly capped containers - up to 3 months at 15-20˚C in the dark.

**References:** Kligler, I.J. (1917). A Simple Medium for the Differentiation of Members of the Typhoid - Paratyphoid Group. Am. J. Publ. Hlth, 7:1042-1044.

Bailey, S.F. and Lacey, G.R. (1927). A modification of the Kligler Lead Acetate Medium. J. Bact. 13:182-189.

## *Lactose Broth*

**LAB126**

**Description**

A medium used for the performance and confirmation of the Pre-sumptive Test for members of the coliform group in water and dairy products.

| Formula | g/litre |
|---|---|
| Beef Extract | 3.0 |
| Gelatin Peptone | 5.0 |
| Lactose | 5.0 |

**Method for reconstitution**

Weigh 13 grams of powder, disperse in 1 litre of deionised water, heat to dissolve then distribute into bottles with Durham tubes. Sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Straw coloured, clear.

**pH:** 6.9 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** See methods for standard techniques.

**Incubation:** 35˚C aerobically for 48 hours.

**Interpretation:** Coliforms are presumptively identified by their ability to ferment lactose and produce gas within 48 hours at 35˚C.

**References**

American Public Health Association. (1975). Standard Methods for the examination of water and waste water, 892. Washington. United States Pharmocopeia, XXI, 1985.

## *Lauryl Tryptose Broth*

***(Lauryl Sulphate Broth, LTB, LSB)***

**LAB196**

**Description**

Lauryl Tryptose Broth is a selective medium for the detection of coliforms in water, dairy products and other foods. The American Public Health Authority (APHA) recommend Lauryl Tryptose Broth for the Most Probable Number Presumptive Test of coliforms in waters, effluent or sewage and as a confirmation test of lactose fermentation with gas production from milk samples and for the detection of coliforms in foods.

Lauryl Tryptose Broth is prepared according to the formulation of Mallmann and Darby. Mallmann and Darby showed that tryptose at a concentration of 2% increased the early logarithmic growth phase when compared to meat peptone. These researchers added phosphate buffers and sodium chloride, which improved gas production by "slow lactose fermenting" organisms. Sodium lauryl sulfate was incorporated as a selective agent for the inhibition of non-coliform organisms.

This medium can also be used with the addition of MUG (4-methylumbelliferyl-β-D-glucuronide) according to the ISO Standard 11866-1 to give enhanced detection of *Escherichia coli*.

| Typical Formula | g/litre |
|---|---|
| Tryptose | 20.0 |
| Lactose | 5.0 |
| Sodium chloride | 5.0 |
| Dipotassium hydrogen phosphate | 2.75 |
| Potassium dihydrogen phosphate | 2.75 |
| Sodium lauryl sulphate | 0.1 |

**Method for reconstitution**

Weigh 35.6 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into tubes or bottles containing inverted Durham tubes. Sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** straw, clear liquid.

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Enterococcus faecalis* WDCM 00087 (inhibition)

**Storage of Prepared Medium:** Store the prepared medium at room temperature (18-22°C), in the dark.

**Inoculation:** Inoculate the medium in accordance with standard methods or laboratory policy.

**Incubation:** 35°C ± 2°C for 24 and 48 hours.

**Interpretation:** After incubation at 35°C for 24 hours examine for turbidity and gas production. If no gas has formed incubate for a further 24 hours and re-examine.

Turbidity in the medium accompanied by the formation of gas within 48 hours is a presumptive result for the presence of coliforms. The results should be confirmed by standard testing methods.

**References**

American Public Health Association (1980) Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.

American Public Health Association (1978) Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.

American Public Health Association (1976) Standard Methods for the Examination of Foods. 15th Edn. APHA Inc. Washington DC.

Mallmann, W.L. and Darby, C.W. (1941) Am. J. Pub. Hlth. 31. 127-134.

ISO Standard 11866-2 Milk and Milk Products –Enumeration of presumptive *Escherichia coli* – part 2: Most probable number technique using 4-methyl umbelliferyl-β-D-glucuronide.

## *Letheen Agar*

***(Tryptone Glucose Extract Agar with Lecithin and Polysorbate 80)***

**LAB185**

**Description**

Letheen Agar is used for evaluating the bactericidal activity of quaternary ammonium compounds, and is used with Letheen Broth to determine the suitability of preservatives for use in cosmetic formulations, as specified by the American Society for Testing and Materials (ASTM), Standard Test Method for Preservatives in Water-Containing Cosmetics. Letheen Agar is a modification of Tryptone Glucose Extract (TGE) Agar, and is formulated to neutralise quaternary ammonium compounds used in testing of germicidal activity, the importance of which was first described by Weber and Black in 1948. The addition of Polysorbate 80 means Letheen Agar also neutralises phenols, hexachlorophene, formalin and ethanol (in the presence of lecithin). Letheen Agar also allows calculation of colony forming units to be assessed, when used with a hygiene swabbing protocol and will ensure against disinfectant carry-over from the swabbing diluent/medium.

| Typical Formula | g/litre |
|---|---|
| Dextrose | 1.0 |
| Tryptone | 5.0 |
| Beef extract | 3.0 |
| Lecithin | 1.0 |
| Polysorbate 80 | 7.0 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 32.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix, and then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes and allow the medium to set.

**Appearance:** Straw, opalescent gel.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* ATCC 11229
*Staphylococcus aureus* ATCC 6538

**Storage of Dehydrated Medium:** Store at 2-8°C in the dark. Formulation is very hygroscopic, keep container tightly closed after use.

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C in the dark.

**Inoculation:** Preservative testing - From the dilutions of product in Letheen Broth, subculture to Letheen Agar using a pour plate technique, or surface inoculation.

Hygiene swabbing – Subculture from the swab diluent using a pour plate or surface inoculation to allow calculation of the colony forming units (cfu) for the area swabbed.

**Incubation:** 37°C aerobically for 24-48 hours.

**Interpretation:** Count all colonies and calculate the number of cfu per ml of sample allowing for dilution factors, or the cfu of the area swabbed (typically 25cm$^2$).

**References**


Weber, G.R. and Black, L.A. (1948). Relative efficiencies of quaternary inhibitors. Soap and Sanit. Chem. 24: 134-139.

American Society for Testing Materials. (1998). Standard Test Method for Preservatives in Water-Containing Cosmetics. E640-78.
Annual Book of ASTM Standards, Philadelphia, PA.

Association of Analytical Chemists. (1995). Official methods of analysis, 16th edition, section 6.Association of Official Analytical Chemists, Washington, D.C.

Roberts, D., Hooper, W., and Greenwood, M. (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.


## *Letheen Broth*

**LAB184**

**Description**

Letheen Broth is primarily used for the assessing the bactericidal activity of quaternary ammonium compounds, and for determining the phenol co-efficient of cationic surfactants as recommended by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC). It is also used in hygiene swabbing protocols where it is necessary to neutralise quaternary ammonium compounds. A modification of FDA Broth, Letheen Broth contains lecithin to neutralise quaternary ammonium compounds and Polysorbate 80 to neutralise phenols, hexachlorophene, formalin and (with lecithin) ethanol. Letheen Broth is easily prepared and has a clear appearance aiding in visual inspection for growth. The American Society for Testing Materials (ASTM) specifies the use of Letheen Broth in the Standard Test Method for Preservatives in Water Containing Cosmetics.

| Typical Formula | g/litre |
|---|---|
| Peptone | 10.0 |
| Beef extract | 5.0 |
| Sodium chloride | 5.0 |
| Lecithin | 0.7 |
| Polysorbate 80 | 5.0 |

**Method for reconstitution**

Weigh 25.7 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix, and then sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Straw, clear liquid.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* ATCC 11229
*Staphylococcus aureus* ATCC 6538

**Storage of Powder:** Store at 2-8°C in the dark. Formulation is very hygroscopic, keep container tightly closed after use.

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** There are a variety of methods which use Letheen Broth and the appropriate references should be consulted. For example:

Phenol co-efficient testing – Subculture from disinfectant dilutions into 10ml volumes of Letheen Broth

Hygiene swabbing – Swab measured area or specific equipment and place in 10ml volume of Letheen Broth. Area to be swabbed and volume of medium may vary depending upon swabbing protocol used.

**Incubation:** 37°C aerobically for 24-48 hours.

**Interpretation:** Examine all tubes for turbidity or as stipulated in the method.

**References**


American Society for Testing Materials, (1998). Standard Test Method for Preservatives in Water-Containing Cosmetics. E640-78.
Annual Book of ASTM Standards, Philadelphia, PA.

Association of Analytical Chemists, (1995). Official methods of analysis, 16th edition, section 6. Association of Official Analytical Chemists, Washington, D.C.

Roberts, D., Hooper, W. and Greenwood, M. (1995). Methods for the examination of food for micro-organisms of public health significance, 2nd edition, section 5.10, Practical Food Microbiology. Butler & Tanner. ISBN 0 901144 36 3.


## *Listeria Enrichment Broth*

**LAB138**

**Description**

A medium for the selective enrichment of food and environmental samples for *Listeria* spp. first described in 1987 by J. Lovett. The medium offers more rapid enrichment than the low temperature enrichment techniques. The medium is incubated at 30˚C and utilises acriflavine, nalidixic acid and cycloheximide as selective agents.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 17.0 |
| Soy Peptone | 3.0 |
| Sodium chloride | 5.0 |
| Dipotassium hydrogen phosphate | 2.5 |
| Glucose | 2.5 |
| Yeast Extract | 6.0 |

**Method for reconstitution**

Weigh 36 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of reconstituted X139. Aseptically dispense into sterile tubes or bottles.

**Appearance:** Yellow, clear.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *L. monocytogenes* WDCM 00021
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 14 days at 2-8˚C in the dark.

**Inoculation:** Add 25 grams of sample to 225mls of Listeria Enrichment Broth and homogenise.

**Incubation:** 30˚C aerobically for up to 48 hours.

**Subculture:** After 24 and 48 hours onto Listeria Isolation Medium – LAB122.

**References**


Lovett, J. Frances, D.W. Hunt, J.M. J. Food Protect. 50: 188-192.
Bolton, F. J. Personal Communication Public Health Laboratory, Preston U.K.

# *LEE Broth*

***Listeria Express Enrichment Broth***

**LAB589**

**Description**

Listeria Express Enrichment Broth (LEE Broth) is a selective enrichment broth for the detection of *Listeria*. Developed to give improved growth rates of *Listeria* over traditional selective enrichment media, LEE Broth enhances the expression of target antigens for most commercially available immunological test kits/methods whilst maintaining adequate suppression of potential non-target organisms. Selective components are blended into the powder, removing the requirement for supplementation.

Compared with more traditional methods, immunological tests, such as ELISA, require relatively high levels of target organisms to achieve a reliable positive result. LEE Broth has been specifically designed to stimulate growth from low numbers to the required high levels within a 24 hour period. Therefore, LEE Broth offers an excellent choice for laboratories employing immunological test methods for detection of *Listeria* in food products.

Lab M LEE Broth can be used as an enrichment broth prior to plating on selective media such as Harlequin™ Listeria Chromogenic Agar ISO (HAL010 / PIN001); as secondary selective enrichment medium following primary enrichment in, for example, Half Fraser Broth (LAB211 or LAB164); or as the enrichment step of a rapid method e.g. ELISA, lateral flow device, PCR.

| Typical Formula | g/litre |
|---|---|
| Peptone | 13.0 |
| Growth enhancers | 8.0 |
| Buffer | 22.2 |
| Selective mix | 3.0 |
| **Grams per litre** | **46.2** |

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, dark straw liquid with yellow fluorescence

**pH:** 7.2 ± 0.1

**Hazard classification:**
Xi – Irritant

**Method for reconstitution:**

Disperse 46.2g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and dispense into suitable containers. Sterilise by autoclaving for 15 minutes at 121°C. Allow to cool to ambient temperature prior to use.

**Incubation:**

Incubate at 30°C ± 1°C for 24 ± 1 hour (for optimum performance check at 24 hours precisely). Alternative incubation temperatures may be used if flagella are not the target antigen.

**Interpretation:**

Low cell numbers may express high levels of detection targets therefore further tests (plating, ELISA etc) should be conducted on all samples regardless of presence/absence of turbidity.

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.
Prepared media: 1 month at 2-8°C in the dark.

Minimum Q.C. organisms:
*Listeria monocytogenes* WDCM 00020
*Listeria ivanovii* WDCM 00018
*Enterococcus faecalis* WDCM 00087 (inhibition)

# *Listeria Isolation Medium (Oxford)*

**LAB122**

**Description**

A selective identification medium for the isolation of *Listeria monocytogenes* from food and clinical material. Columbia agar is the nutrient base to which selective inhibitors have been added. Lithium chloride is used to inhibit enterococci and acriflavine to inhibit some Gram negative and Gram positive species. Further selective agents may be added after autoclaving to increase the selectivity; these are colistin, fosfomycin, cefotetan and cyclohexamide. Aesculin is included in the formula as a differential indicator. *L. monocytogenes* will hydrolyse aesculin to aesculutin which reacts with the iron salt to give a black precipitate around the colonies.

Lab M's formulation has been used to successfully isolate *Listeria* from such diverse products as chicken giblets and dairy cheeses. The advisability of using this medium at two levels of selectivity has been recognised.

| Typical Formula | g/litre |
|---|---|
| Columbia Agar Base | 41.0 |
| Aesculin | 1.0 |
| Ferric ammonium citrate | 0.5 |
| Lithium chloride | 15.0 |

**Method for reconstitution**

Weigh 57.5 grams of powder. Add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 47˚C, add 2 vials of selective supplement X123 mix well and pour plates.

**Appearance:** Pale yellow, slightly opaque gel.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *L. monocytogenes* WDCM 00021
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streak out to single colonies. This medium is highly selective, a heavy inoculum can be used.

**Incubation:** 30˚C aerobically for 24-48 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *L. monocytogenes* | 0.5-1.0 | CV.E.G. | Grey/ Green | Black/brown around colonies diffusion |
| Enterococci | p.p. - 0.5 | CV.E.G. | Black | Usually no growth |

**References**


Garayzabal, J.F.F., Rodriguez, L.D., Boland, J.A.V., Cancelo, J.L.B., Fernandez, G.S. (1986). *Listeria monocytogenes* dans le lait pasteurise. Can. J. Microbiol. 32: 149-150.

Donnelly. C.W., Gregory J. Baigent (1986). Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl. & Environ. Microbiol.Oct. 689-695.

Bolton, C.F.J. Preston P.M.L. Personal communication. Lovett, J. Francis, D.W. Hunt. J.M. (1987). *Listeria monocytogenes* in raw milk: Detection, Incidence and Pathogenicity. Journ. Food Protect. Vol. 50. No. 3: 188-192.

Van Netten, P., Van de Van, A., Perales, I., Mossel, P.A.A. (1988). A selective and diagnostic medium for use in enumeration of *Listeria* spp. in foods. International Journal of Food Microbiology 6:187-198.

# *Listeria Isolation Media*
***(Oxford Formulation)***

**LAB206**

**Description**

A selective identification medium for the isolation of *Listeria monocytogenes* from food and clinical material. Columbia agar is the nutrient base to which selective inhibitors have been added. Lithium chloride is used to inhibit Enterococci, whilst acriflavine inhibits some Gram-negative and Gram-positive species. Further selective agents may be added after sterilisation to increase the selectivity.

| Typical Formula | g/litre |
|---|---|
| Columbia agar base | 41.0 |
| Aesculin | 1.0 |
| Ferric ammonium citrate | 0.5 |
| Lithium chloride | 15.0 |

**Method for reconstitution**

Weigh 57.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C, and add 2 vials of X123. Mix well before dispensing into sterile Petri dishes.

**Appearance:**
Powder: Fine, free-flowing, homogeneous, buff.

Final medium: Straw-yellow gel.

**pH:** 7.0 ± 0.2

**Hazard classification**
Xn - Harmful

Minimum Q.C. organisms:
*Listeria monocytogenes* WDCM 00021
*Escherichia coli* WDCM 00013
*Enterococcus faecalis* WDCM 00087

**Storage:**

Dehydrated culture media: 10-25°C

Poured plates: 7 days at 2-8°C in the dark

**Inoculation:** Surface inoculation as per user's validated methods.

**Incubation:** Incubate for 24-48 hours at 30, 35 or 37°C according to user's validated methods.

**References**

ISO 11290-1:1996 Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method.

# *Listeria Monocytogenes Blood Agar*
***(LMBA)***

**LAB172**

**Description**

*Listeria monocytogenes* Blood Agar (LMBA) has been developed for the specific detection and enumeration of *L. monocytogenes* in food samples. This medium has been shown to improve the isolation rate of *L. monocytogenes* in ready to eat foods by up to 22%.

*L. monocytogenes* is the only important human pathogen among the species of Listeria currently recognised. It is distinguished from *L. innocua* on LMBA using colonial appearance and haemolysis. Studies have shown that the most commonly isolated species of listeria, other that *L. monocytogenes* from food and processing environments is *L. innocua*.

*L. monocytogenes* can be found in all main categories of products e.g. dairy, meat and poultry. The symptoms of infection with this organism include fever, generalised aches and pains, sore throat, diarrhoea and abdominal pains. In severe cases pneumonia, septicaemia and meningitis may develop. Pregnant women are particularly susceptible to listeriosis, due to immune suppression. *L. monocytogenes* can cross the placenta causing abortion, still birth, or meningitis of the new born.

LMBA contains lithium chloride in concentrations that inhibit the growth of enterococci yet allow good haemolysis by *L. monocytogenes*. LMBA is supplemented by polymyxin plus ceftazidine (X072) and nalidixic acid (X072N) to suppress competing flora such as members of the bacillus group and staphylococci.

The addition of donated sheep blood (defibrinated with sodium citrate) to LMBA allows differentiation between haemolytic and non-haemolytic stains of *Listeria*. The use of sheep blood is standard methodology for *Listeria* testing. However, ingredients in selective agars can result in partial lyses or darkening of the blood supplement. The use of citrated sheep blood prevents this and allows differentiation of *L. monocytogenes* from other haemolytic *Listeria* species e.g. *L. seeligeri* and *L. ivanovii*, due to its distinctive haemolytic pattern. *L. seeligeri* is rarely isolated from foods and produces very weak haemolysis, whilst *L. ivanovi*i produces wide zones of haemolysis compared to the narrow zone of *L. monocytogenes* and is an animal rather than human pathogen.

LMBA is a cost effective method by which to specifically isolate and enumerate *L. monocytogenes*

| Typical Formula | g/litre |
|---|---|
| Tryptone | 15.0 |
| Soy peptone | 5.0 |
| Sodium chloride | 5.0 |
| Lithium chloride | 10.0 |
| Magnesium sulphate (3/4$H_2O$) | 3.8 |
| Agar | 15.0 |

**Method for Reconstitution**

Weigh 53.8 grams of powder and disperse into 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix then sterilise at 121°C for 15 minutes. Cool to 47°C, and aseptically add citrated sheep blood to 5%, 2 vials of X072 supplement and 2 vials of X072N supplement. Mix well, and pour into sterile Petri dishes and allow to set.

**Appearance:** Opaque and blood red.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms:
*Listeria monocytogenes* WDCM 00021
*Listeria innocua* WDCM 00018
*Escherichia coli* WDCM 00013

**Storage of Prepared Medium:** Plates can be stored up to 7 days at 2-8°C in the dark.

**Inoculation:** Surface plating, streaking out to single colonies, from the enrichment broth. For enumeration, 0.1ml of neat or 10-1 dilution of the food sample is spread over the entire surface of the plate. Use multiple plates if the volume required is greater than 0.1 ml.

**Incubation:** Aerobically at 37°C for 24-48 hours.

**Interpretation:**

| Organism | Shape and Surface | Colour & Haemolysis |
|---|---|---|
| *L. monocytogenes* | C.V.E.G. | Cream, narrow zone of β haemolysis |
| *L. ivanovii* | C.V.E.G. | Cream, wide zone of β haemolysis |
| *L. innocua* | C.V.E.G. | Cream, no haemolysis |
| *L. seeligeri* | C.V.E.G. | Cream, very weak β haemolysis |
| *Enterococcus faecalis* | | No growth |
| *Escherichia coli* | | No growth |

**References**

Johansson, T (1998). Enhanced detection and enumeration of *Listeria monocytogenes* from foodstuffs and food processing environments. International Journal of Food Microbiology, 40; 77-85.

Jay, J.M. (1996). Prevalence of *Listeria* spp. in meat and poultry products. Food Control, 7; 209-214.

Kozak, J., Balmer, T., Byrne, R. and Fisher, K. (1996). Prevalence of *Listeria monocytogenes* in foods: incidence in dairy products. Food Control, 7; 215-222.

# *Lysine Agar*

**LAB201**

**Description**

Originally described by Morris and Eddy, this complex synthetic medium is designed for the isolation and enumeration of wild yeasts in pitching yeast. Lysine is utilised by wild yeasts, but not by *Saccharomyces cerevisiae*, *S. carlsbergensis* and *S. pastorianus*. Lab M Lysine Agar is made to the Morris and Eddy published formulation.

| Typical Formula | g/litre |
|---|---|
| Dextrose | 4.0 |
| Lysine Agar Chemical | 4.0 |
| Agar | 1.0 |

**Method for reconstitution:**

Disperse 6.6g of powder in 100ml of distilled water and add 1 vial (1ml) of X034, Poptassium lactate. Allow to soak for 10 minutes, swirl to mix and bring to the boil with frequent agitation to prevent superheating. Cool to 50oC and add 0.1ml of X036, 10% Lactic Acid. Mx well and dispense into sterile petri dishes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: colourless clear gel

**pH:** 4.8 ± 0.2 (complete medium)

**Hazard classification:**
NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C
Final medium: 7 days at 2-8°C in the dark

Minimum Q.C. organisms:
*Pichia fermentans* NCYC 850
*Saccharomyces pastorianus* NCYC 185

# *Lysine Iron Agar*

**LAB054**

**Description**

This is a differential medium for the detection of salmonellae and other enteric pathogens, by means of lactose fermentation, lysine decarboxylase activity and hydrogen sulphide production. *Salmonella* strains (including *Salmonella arizona*) which ferment lactose and produce black colonies on Bismuth Sulphite Agar (LAB013) can be recognised by the alkaline reaction (purple colour) produced throughout the medium, together with blackening due to sulphide production. Enteric organisms that do not decarboxylate lysine yield an alkaline slant over an acid butt (yellow). Thus no distinction between *Shigella* and *E. coli* is possible and Triple Sugar Iron Agar (LAB053) is recommended in parallel. *Proteus* and *Providencia* cultures characteristically produce a distinctive red slant over an acid butt since these organisms deaminate lysine but without sulphide production. *Salmonella arizona* strains which produce pink to red colonies on bile salt media are often overlooked in outbreaks of food poisoning, however the use of Bismuth Sulphite Agar with subculture into Lysine Iron Agar allows determination of their presence.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 5.0 |
| Yeast Extract | 3.0 |
| Glucose | 1.0 |
| L-Lysine | 10.0 |
| Ferric Ammonium Citrate | 0.5 |
| Sodium thiosulphate | 0.04 |
| Bromocresol Purple | 0.02 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 31.5 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and bring to the boil, with frequent stirring to dissolve completely. Dispense into tubes and sterilise by autoclaving at 121°C for 15 minutes. Cool in a slanted position such that slopes are formed over deep butts approx. 3cm in depth.

**Appearance:** Clear purple gel.

**pH:** 6.7 ± 0.2

**Storage of Prepared Medium:** Tightly capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Subcultures for further identification are picked from the centre of isolated colonies on selective media and streaked across the slant and stabbed into the butt of tubes of Lysine Iron Agar.

**Incubation:** 37°C aerobically for 18-24 hours.

**Growth Characteristics**

| Organism | Butt | Slant | Sulphide Production |
|---|---|---|---|
| *Salmonella* | Alkaline | Alkaline | + |
| *Enterobacter aerogenes)* | | | |
| *Citrobacter* | Acid | Alkaline | + |
| *Escherichia coli* | Acid (NC) | Alkaline | - |
| *Shigella* | Acid | Alkaline | - |
| *Proteus* | Acid | 'red' | - |

**References**

Edwards, P.R. and Fife, M.A. (1961). Lysine iron agar in the detection of Arizona cultures. Appl. Microbiol. 9:478-480.

Edwards, P.R. and Ewing, W.H. (1964). Identification of Enterobacteriaceae. Burgess Publishing Co. Minn.

# *M17 Agar*

**LAB092**

**Description**

A medium for the enumeration of Lactococci in dairy products. The medium can also be used to investigate the bacteriophage susceptibilities of these organisms. Another application is for the enumeration of *Streptococcus thermophilus* in yoghurts.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone | 5.0 |
| Soy Peptone | 5.0 |
| Yeast Extract | 2.5 |
| Beef Extract | 5.0 |
| Lactose | 5.0 |
| Sodium glycerophosphate | 19.0 |
| Magnesium sulphate | 0.25 |
| Ascorbic acid | 0.5 |
| Agar No. 2 | 15.0 |

**Method for reconstitution**

Weigh 57.2 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to boil to dissolve agar before dispensing in 15ml aliquots. Sterilise by autoclaving at 115˚C for 20 minutes.

**Appearance:** Pale straw, translucent agar.

**pH:** 7.1 ± 0.2

Minimum Q.C. organisms: *Lactococcus lactis* WDCM 00016

**Storage of Prepared Medium:** Capped containers – up to 1 month at 15-20˚C in the dark.

**Inoculation:** Pour plate technique.

**Incubation:** 30˚C for 48-72 hours for mesophilic streptococci, 37˚C for 48 hours for *Streptococcus thermophilus.*

**Interpretation:** Count all colonies. Streptococci form colonies of 1-2mm in diameter.

**References**

Terzaghi, B.E. Sandine, W.E.. (1975). Improved medium for lactic Streptococci and their Bacteriophages. Appl. Microbiol. 29 No. 6 pp 807-813.

# *MacConkey Agar*

***(With Salt)***

**LAB030**

**Description**

A selective medium for the isolation of bile tolerant organisms from faeces, urine, sewage and foodstuffs. Bile-tolerant Gram positive organisms as well as Gram negative organisms will grow on this medium. This formula is recommended by W.H.O. and other bodies for the examination of water and milk. Some strains of *Proteus* spp. will spread on this medium making interpretation difficult, for this reason LAB002 MacConkey Agar (without salt) may be preferred as it is less prone to this phenomenon.

| Typical Formula | g/litre |
|---|---|
| Peptone | 20.0 |
| Lactose | 10.0 |
| Bile Salts | 5.0 |
| Sodium chloride | 5.0 |
| Neutral red | 0.05 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 52 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and mix well before pouring into Petri dishes.

**Appearance:** Pink/red, clear

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface plating, streaking out to single colonies.

**Incubation:** 37˚C aerobically for 24 hours.

**Growth Characteristics**

| organism | approx. size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Proteus* spp. | 1.5-2.5 | CV.E.G. (spreading) | Yellow | |
| *Salmonella* spp. | 1.5-2.5 | CV.E.G. | Colourless | |
| *S. aureus* | 0.5-2.0 | CV.E.G. | White/ Pink<br>Orange<br>Opaque | (dependent on lactose fermentation and pigment production) |
| *Enterococcus* spp. | P.P.-0.5 | CV.E.G. | Pink/Deep Red Opaque | |

**References**

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

World Health Organisation (1971). International Standards for Drinking Water. 3rd Edn. W.H.O., Geneva.

Taylor, E.W. (1958). The Examination of Water and Water Supplies. 7th Edn. Churchill, London.

Cruikshank, R. (1973). A Guide to the Laboratory Diagnosis and Control of Infection. Medical Microbiology. 12th Edn. Churchill.

# *MacConkey Agar*

***(without salt)***

**LAB002**

**Description**

A medium first introduced by MacConkey in 1905 for the isolation and differentiation of lactose and non lactose fermenting enteric bacteria. The medium has since been modified to improve the recovery of staphylococci and enterococci, it is used for culturing a wide range of clinical material and has applications in food, water and dairy bacteriology.

| Typical Formula | g/litre |
|---|---|
| Mixed Peptones | 20.0 |
| Lactose | 10.0 |
| Bile | 5.0 |
| Neutral red | 0.05 |
| Agar No. 2 | 13.5 |

**Method for reconstitution**

Weigh 48.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C and mix well before pouring plates. Prior to inoculation, dry the surface of the agar by partial exposure at 37˚C.

**Appearance:** Pink/red, clear.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*S. aureus* WDCM 00034

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface inoculation, streaking for single colonies.

**Incubation:** 37˚C aerobically for 24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *E. coli* | 2.0-3.0 | CV.E.G. | Red | (non lactose fermenting yellow) |
| *Proteus* spp. | 2.0-3.0 | CV.E.G. | Yellow | fishy odour |
| *Salmonella* spp. | 2.0-3.0 | CV.E.G. | Yellow | |
| *S. aureus* | 0.5-1.0 | CV.E.G. | Pink-Orange | (lactose-negative) |
| *Enterococcus* spp. | 0.5 | CV.E.G. | Pink-Deep Red | |

**References**


MacConkey, A.T. (1905) Lactose-fermenting bacteria in faeces. J.Hyg. (Camb), 5: 333-379.

MacConkey, A. T. (1908) Bile salt media and their advantages in some bacteriological examinations, J.Hyg. (Camb.), 8: 322-341.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

World Health Organisation (1971), International Standards for Drinking Water, 3rd Edn. W.H.O., Geneva. Taylor, E.W. (1958). The Examination of Water Supplies, 7th Edn. Churchill, London


# *MacConkey Agar No.2*

**LAB216**

**Description**

MacConkey Agar No.2 is a modification of MacConkey Agar which contains bile salts No. 2 for the recognition of enterococci. This is especially useful when looking for enterococci in the presence of coliforms and non-lactose fermenters from water, sewage and food products. Enterococci are frequently sought as an index of faecal pollution and appear on this medium as small, intensely coloured red-purple colonies. Non-lactose fermenters appear colourless, whilst bile tolerant Gram-positive organisms, such as staphylococci and non-faecal streptococci, are completely inihibited.

| Typical Formula | g/litre |
|---|---|
| Peptone | 20.0 |
| Lactose | 10.0 |
| Bile salts No.2 | 1.5 |
| Sodium chloride | 5.0 |
| Neutral red | 0.05 |
| Crystal violet | 0.001 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 51.6 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, red-purple gel

**pH:** 7.2 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Enterococcus faecalis* WDCM 00087
*Escherichia coli* WDCM 00013
*Salmonella typhimurium* WDCM 00031
*Staphylococcus aureus* WDCM 00034 (inhibited)

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** Surface inoculation as per user's validated methods.

**Incubation:** Incubate at 37°C + 1°C for 18 - 48 hours.

**Interpretation:** After incubation the plate should be assessed for typical colonies.

**Interpretation**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *Enterococcus faecalis* | 0.5mm | Convex, entire, glossy | Red-purple |
| *Escherichia coli* | 3 - 4mm | Convex, entire, glossy | Red-purple |
| *Salmonella* spp. | 2 – 3mm | Convex, entire, glossy | Translucent |
| *Staphylococcus aureus* | No growth | | |

# *MacConkey Agar No. 3*

**LAB045**

**Description**

A modification recommended by the W.H.O. and the American Public Health Association for the isolation of *Enterobacteriaceae* from waters and sewage. The medium has been made more selective than MacConkey's original formula by the use of crystal violet as well as bile salts. Gram positive organisms will not grow on this medium.

| Typical Formula | g/litre |
|---|---|
| Peptone | 20.0 |
| Lactose | 10.0 |
| Bile Salts No. 3 | 1.5 |
| Sodium chloride | 5.0 |
| Neutral red | 0.03 |
| Crystal violet | 0.001 |
| Agar No. 2 | 15.0 |

**Method for reconstitution**

Weigh 51.5 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 47˚C and pour into Petri dishes. Dry the surface before inoculation.

**Appearance:** Pale red slight violet tinge.

**pH:** 7.1 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00031
*Ent. faecalis* (inhibition) WDCM 00087

**Storage of prepared medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking for single colonies.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *E. coli* | 3.0-4.0 | CV.E.G. (D) | Red | (red ppt around colony) |
| *Proteus* spp. | 3.0-4.0 | CV.E.G. | Pale-yellow | (fishy odour) |
| *Staph. aureus* | No growth | | | |
| *Enterococcus* spp. | No growth | | | |

**References**

American Public Health Association (1950). Diagnostic Procedures and Reagents. 3rd edn. A.P.H.A., New York.

American Public Health Association (1946). Standard Methods for the examination of Water and Sewage. 9th edn. A.P.H.A., New York

# *MacConkey Broth*

***(Purple)***

**LAB005**

**Description**

This medium is used in the detection and enumeration of faecal coliforms (37˚C) and *E. coli* (44˚C). The replacement of neutral red used in the original formulation by bromocresol purple makes the colour change caused by acid producing organisms easier to read.

| Typical Formula | g/litre |
|---|---|
| Peptone | 20.0 |
| Lactose | 10.0 |
| Bile Salts | 5.0 |
| Bromocresol purple | 0.01 |

**Method for reconstitution**

Weigh 35 grams of powder, disperse in 1 litre of deionised water. Mix well and dispense into tubes or bottles with inverted Durham tubes. Sterilise by autoclaving for 15 minutes at 121˚C. Prepare double strength broth (70g/l) if 50ml or 10ml amounts of inoculum are to be added to equal volumes of broth. Prepare single strength broth (35g/l) if 1ml or 0.1ml amounts of inoculum are to be added to 10ml of broth.

**Appearance:** Purple, clear.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*B. subtilis* WDCM 00070

**Storage of Prepared Medium:** Capped containers – up to 1 month at 15-20˚C in the dark.

**Incubation:** 37˚C aerobically for coliforms, 44˚C aerobically for *E. coli*. Use Durham tubes to detect gas production for *E. coli*.

**Growth Indicators:** Turbidity, gas production. Lactose-fermenting organisms cause a colour change from purple to yellow.

**References**

Ministry of Health (1937). Bacteriological Tests for Graded Milk, Memo 139/Foods. H.M.S.O., London.

Minister of Health, Public Health Laboratory Service Water Committee (1969). The Bacteriological Examination of Water Supplies, 4th Edn. report No. 71. H.M.S.O., London.

World Health Organisation (1971). International Standards for Drinking Water, 3rd Edn, W.H.O., Geneva.

# *Malt Extract Agar*

**LAB037**

**Description**

An acidic medium which will support the growth of most yeasts and moulds whilst inhibiting most bacteria. It was first described by Thom and Church in 1926 in a study of *Aspergillus* spp. claiming the high carbohydrate content ensured rapid growth. Selectivity can be increased by further lowering the pH with the addition, after sterilisation, of X037 Lactic Acid. It should be noted that excess heating of this medium together with its low pH can easily result in hydrolysis of the agar gel producing soft plates.

| Typical Formula | g/litre |
|---|---|
| Malt Extract | 30.0 |
| Mycological Peptone | 5.0 |
| Agar No. 2 | 15.0 |

**Method for reconstitution**

Weigh 50 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise at 115˚C for 10 minutes. If the addition of X037 Lactic Acid is required this should be done after sterilisation. One 5ml vial of X037 will lower the pH of 250ml of medium to 3.5-4.0. Cool to 47˚C before making additions and pouring plates.

**Appearance:** Pale brown/straw, clear.

**pH:** 5.4 ± 0.2 (if X037 is added pH 3.5-4.0)

Minimum Q.C. organisms: *Candida* spp. WDCM 00054

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 1 month at 15-20˚C in the dark.

**Inoculation:** Pour plate technique or surface streaking for single colonies.

**Incubation:** 25˚C aerobically for 5 days.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Candida albicans* | 4 | CV.E.D. | White | |
| *Candida krusei* | 10 | F.CR.D. | White | |
| *Penicillium notatum* | 25 | | Green velvet | (white/yellow - strain dependent) |
| *Aspergillus niger* | 25 | | White border, black centre | (yellow/black centre) |

**References**

Galloway, L.D. and Burgess, R. (1952). Applied Mycology and Bacteriology, Leonard Hill, London. Thom and Church, 1926. The Aspergilli.

# *Malt Extract Broth*

**LAB159**

**Description**

A liquid medium of low pH for the growth of yeasts and moulds, typically employed as part of sterility testing protocols for various products. The high carbohydrate content of the medium ensures rapid growth of yeasts and moulds.

| Typical Formula | g/litre |
|---|---|
| Malt Extract | 17.0 |
| Mycological Peptone | 3.0 |

**Method for reconstitution**

Weigh 20g of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to dissolve and dispense into final containers. Sterilise by autoclaving at 115˚C for 10 minutes.

**Appearance:** Pale brown/straw, clear broth.

**pH** 5.4 ± 0.2

**Inoculation:** Inoculate samples direct into tubes of broth according to the particular method being employed.

**Incubation:** 25˚C (or 37˚C) for up to 7 days aerobically, depending upon protocol used. Subculture turbid tubes onto solid media for identification of growth.

Minimum QC organism: *Candida albicans* WDCM 00054
*Aspergillus niger*

**References**

Galloway, L.D. and Burgess, R. (1952) Applied Mycology and Bacteriology, 3rd ed, Leonard Hill, London pp 54 & 57.

# *Mannitol Salt Agar*

**LAB007**

**Description**

Mannitol Salt Agar is a medium for *Staphylococcus aureus* which is selective because the high sodium chloride level inhibits most other species with the exception of halophilic Vibrios. The majority of *S. aureus* ferment mannitol producing yellow colonies, occasional strains of coagulase-negative staphylococci may also ferment mannitol. It is necessary to confirm the identity of presumptive *S. aureus* colonies by other means e.g. coagulase, protein A, DN'ase, thermonuclease or latex agglutination. This medium performs as per the requirements of the Harmonised Pharmacopoeia (USP/EP/JP).

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 1.0 |
| Balanced Peptone No. 1 | 10.0 |
| Sodium chloride | 75.0 |
| D-Mannitol | 10.0 |
| Agar No. 2 | 12.0 |
| Phenol Red | 0.025 |
| **Grams per litre** | **108.0** |

**Method for reconstitution**

Weigh 108 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121ºC. Cool to 47°C before pouring into Petri dishes.

**Appearance:**

**Powder:** fine, free-flowing, homogeneous, buff

**Finished medium:** clear, red gel

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus ATCC 6538*
*E. coli ATCC 8739* (inhibition)

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation Method:** Surface plating, streak out for single colonies.

**Incubation:** In accordance with Harmonized Pharmacopoeia (USP/EP/JP), incubate aerobically at 30-35ºC for 18-72 hours. The product may also be used for other applications at 37ºC for 48 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. aureus* | 1.5-2.0 | CV.E.G. | Bright Yellow | |
| Other Staphylococci | 1.0-1.5 | CV.E.G. | White or Yellow | (some ferment mannitol) |

*Enterobacteriaceae* no growth


**References**

American Public Health Association (1966). Recommended Methods for Microbiological Examination of Foods, 2nd Edn. (ed. J.M. Sharf) A.P.H.A. Washington.

Davis, J.G., (1959). Milk Testing 2nd edn, Dairy Industries, London.


## *Maximum Recovery Diluent*

***(Peptone/Saline diluent)***

**LAB103**

**Description**

An osmotically controlled solution which is an alternative to, and a replacement for, 1/4 strength Ringer's Tablets (LAB100Z). The presence of a low level of peptone lessens the physiological shock normally experienced by bacterial cells when they are introduced to a diluent such as Ringer's Solution. The level of peptone is such that multiplication of the organisms is not possible in the time in which the sample will be present in the diluent (1-2 hours). This formula is recommended by ISO 6887: BS5763.

| Typical Formula | g/litre |
|---|---|
| Peptone | 1.0 |
| Sodium chloride | 8.5 |
| **Grams per litre** | **9.5** |

**Method for reconstitution**

Weigh 9.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and if required, heat gently to dissolve. Distribute into final containers. Sterilise by autoclaving for 15 minutes at 121ºC.

**Appearance:**

**Powder:** fine, free-flowing, homogeneous, buff

**Finished medium:** clear, colourless liquid

**pH:** 7.0 ± 0.2

N.B. The absence of any buffer results in the product taking on the pH of any sample added.

Minimum Q.C. organisms: *E. coli* WDCM 00031
*S. aureus* WDCM 00034

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.


**References**

Straka, R.P. and Stokes, J.L. (1957). Rapid destruction of bacteria in commonly used diluents and its elimination. Appl. Microbiol. 5: 21-25.

ISO 6887-1:1999 Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions.


## *Membrane Lauryl Sulphate Broth*

**LAB082**

**Description**

This medium superseded Membrane Enriched Teepol broth when Shell Chemicals withdrew Teepol 610 from sale. Sodium lauryl sulphate was found to be an adequate reproducible substitute and this medium is recommended for the enumeration of coliform and organisms in water and sewage.

| Typical Formula | g/litre |
|---|---|
| Peptone | 39.0 |
| Yeast Extract | 6.0 |
| Lactose | 30.0 |
| Phenol red | 0.2 |
| Sodium lauryl sulphate | 1.0 |

**Method for reconstitution**

Weigh 76.2 grams of powder, disperse in 1 litre of deionised water. Distribute into screw cap containers and sterilise by autoclaving at 115˚C for 10 minutes.

**Appearance:** Red, clear solution.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** *E. coli* and coliform counts should be made on separate samples of water. The volumes should be chosen so as the number of colonies on the membrane lies between 10 and 100. With waters expected to contain less than 1 coliform per ml, a sample of 100ml should be filtered. The membrane filter should be placed face upwards on a pad soaked in Membrane Lauryl Sulphate Broth, after filtration. These membranes should be incubated in a container which does not allow evaporation to occur. Water tight metal containers placed in an accurate water bath are required for incubation of membranes at 44˚C.

**Incubation:** *E. coli* 4 hours at 30˚C 14 hours at 44˚C; Coliforms 4 hours at 30˚C 14 hours at 35˚C.

**Interpretation:** No colonies:- assume a nil count. Small colonies of an intermediate colour:- return to incubation for a full period.

*E. coli:* Yellow-coloured colonies from membranes incubated at 44˚C should be subcultured to Lactose Broth LAB126 and Tryptone Water, LAB129 to confirm gas and indole production respectively, after 24 hours incubation at 44˚C.

Coliform organisms: Yellow colonies from membranes incubated at 35˚C or 37˚C should be subcultured into Lactose Broth LAB126. After 48 hours incubation at 37˚C a result should be obtained regarding the production of gas.

Full details of the methodology can be found in The Bacteriological Examination of Water Supplies 71, 1969.


**References**

Burnham, N.P. (1967). Proc. Soc. Wat. Treat, Exam. 16:40.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

Windle Taylor, E. (1961) Glutamic acid medium, 40th Ann Rep. Div. Water Exam. Met. Water Board London pp 18-22.

# *Microbial Content Test Agar (MCA)*

***(Tryptone Soy Agar with Lecithin and Polysorbate 80 (TSALT))***

***(Casein Soy Peptone Agar with Lecithin and Polysorbate 80)***

**LAB189**

**Description**

The use of Microbial Content Test Agar (MCA) is recommended for the detection of microorganisms on surfaces sanitised with quaternary ammonium compounds, phenolic compounds and formalin. The medium is a modification of Tryptone Soy Agar with added neutralising compounds lecithin and Polysorbate 80. It is recommended for determining the hygiene status of containers, equipment and work areas treated with disinfectants or other sanitisers. The addition of Lecithin and Polysorbate 80 in the formula inactivates some preservatives that may inhibit bacterial growth, reducing "preservative carryover". The formulation is recommended for Aerobic Plate Count (Microbial Limit Test) for water miscible cosmetic products containing preservatives. Lecithin is included to neutralise quaternary ammonium compounds and Polysorbate 80 is incorporated to neutralise phenols, hexachlorophene, formalin and with lecithin, ethanol.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 15.0 |
| Soy Peptone | 5.0 |
| Sodium Chloride | 5.0 |
| Polysorbate 80 | 5.0 |
| Lecithin | 0.7 |
| Agar No.2 | 15.0 |

**Method for Reconstitution**

Weigh 45.7 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes and allow the medium to set.

**Appearance:** Straw opalescent gel.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* ATCC 11229
*Staphylococcus aureus* ATCC 6538

**Storage of Powdered Medium:** Store at 2-8°C in the dark. Formulation is very hygroscopic, keep container tightly closed after use.

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C in the dark.

**Inoculation:** Consult the appropriate references as this product is used in several procedures.

**Incubation:** 37°C aerobically for 24-48 hours.

**Interpretation:** Count all colonies and calculate the number of colony forming units, (cfu) per ml of sample allowing for dilution factors.

**References**

Orth, D.S. (1993) Handbook of Cosmetic Microbiology. Marcel Dekker, Inc., New York, NY.

Brummer, B. (1976). Influence of possible disinfectant transfer on Staphylococcus aureus plate counts after contact sampling. App. Environ. Microbiol. 32:80-84.

# *Milk Agar*

**LAB019**

**Description**

An approved formulation for the enumeration of micro-organisms in milk, rinse waters and dairy products. With the addition of a further 5 g/l Agar No. 1 the medium is suitable for the preparation of Roll-Tubes using established mechanical equipment. Also see Milk Plate Count Agar LAB115. Milk Agar can also be used with the P-INC supplement (X019, X219) for accelerated shelf-life determination of dairy products.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 3.0 |
| Peptone | 5.0 |
| Antibiotic Free Skim Milk Powder | 1.0 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 24 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 15 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 45˚C before mixing with sample dilutions.

**Appearance:** White, opalescent gel.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*S. aureus* WDCM 00034

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Pour plate technique.

**Incubation:** Aerobically at 30˚C for 72 hours.

**References**

Ministry of Health (1937). Bacteriological Tests for Graded Milk. Memo 139/Foods H.M.S.O., London. British Standard 4285: Methods of Microbiological Examination for Diary Purposes.

# *Milk Plate Count Agar*

**LAB115**

**Description**

A medium recommended by the British Standards Institute and the International Organisation for Standardisation for the enumeration of viable bacteria in milk and other dairy products.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 5.0 |
| Yeast Extract | 2.5 |
| Dextrose | 1.0 |
| Antibiotic Free Skim Milk Powder | 1.0 |
| Agar No. 1 | 10.0 |

**Method for reconstitution**

Weigh 19.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil to dissolve the agar. Allow to cool to 47˚C and dispense into suitable containers. Sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Pale cream, opalescent gel.

**pH:** 6.9 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*S. aureus* WDCM 00034

**Storage of Prepared Medium:** Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Pour plate technique.

**Incubation:** 30˚C aerobically for 72 hours.

**Interpretation:** Count all colonies. Calculate back to determine viable organisms per ml

**References**

British Standards Institute. (1984). BS 4285 Section 1.2. International Organisation for Standardisation Draft International Standard. (1982) ISO/DIS 6610. D.I.N. 10192.

## *Minerals Modified Glutamate Medium*

**LAB080A & LAB080B**

**Description**

This medium was developed for use with the Most Probable Numbers Technique (M.P.N.) for the enumeration of coliforms in water supplies. The medium is an improved version of the chemically defined glutamic acid medium described by Gray in 1964. The product is supplied in two parts because it has been shown that separating the sodium glutamate from the base improves its stability.

| Typical Formula | g/litre |
|---|---|
| **LAB080A** | **(double strength)** |
| Lactose | 20.0 |
| Sodium formate | 0.5 |
| L-Cystine | 0.04 |
| L(-) Aspartic acid | 0.048 |
| L(+) Arginine | 0.04 |
| Thiamine | 0.002 |
| Nicotinic acid | 0.002 |
| Pantothenic acid | 0.002 |
| Magnesium sulphate ($MgSO_4.7H_20$) | 0.2 |
| Ferric ammonium citrate | 0.02 |
| Calcium chloride ($CaCl_2.2H_2O$) | 0.02 |
| Dipotassium hydrogen phosphate | 1.8 |
| Bromocresol purple | 0.02 |
| **LAB080B** | |
| Glutamic acid (sodium salt) | 12.7 |

**Method for reconstitution**

**Double strength:** Dissolve 22.7 grams of base medium (LAB080A) together with 12.7 grams of sodium glutamate (LAB080B) in 1 litre of deionised water containing 5 grams of ammonium chloride eg BDH cat no. 27149. Dispense 10ml and 50ml volumes into tubes with inverted Durham tube.

**Single strength:** Dissolve in 11.35 grams of base medium (LAB080A) together with 6.35 grams of sodium glutamate (LAB080A) in 1 litre of distilled water containing 2.5 grams ammonium chloride. Dispense 5ml volumes into tubes with inverted Durham tubes.

**Sterilise** by autoclaving for 10 minutes at 115˚C, alternatively heat to 100˚C for 30 minutes on three successive days.

**Appearance:** Purple, clear solution.

**pH:** 6.7 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Use the Most Probable Number technique. With 10ml and 50ml of sample add to equal volumes of double strength medium. With 1ml volumes of sample add to 5ml of single strength medium. Ensure the Durham tube is free of bubbles.

**Incubation:** 37˚C for 18-24 hours aerobically.

**Interpretation** Tubes showing the production of acid (medium turns yellow) and gas in the Durham's tube are considered presumptive positive. Each presumptive positive tube should be subcultured to Brilliant Green Bile Broth LAB051 with Durham tube and incubated at 44˚C for 24 hours and examined for gas production. A tube of Tryptone Water LAB129 should also be inoculated and incubated at 44˚C for 24 hours for the production of indole. The production at 44˚C of gas from lactose and the formation of indole are evidence of *E. coli*.

**References**

Gray, R.D. (1964). An improved formate lactose glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water. J. Hyg. Camb. 62: 495-508.

PHLS Water Sub-Committee. (1958). A comparison between MacConkey broth and Glutamic acid media for the detection of coliform organisms in water. J. Hyg. Camb. 56: 377-388.

PHLS Standing Committee on Bacteriological Examination of Water Supplies. (1968). Comparison of MacConkey Broth, Teepol Broth and Glutamic Acid Media for the enumeration of Coliform organisms in water. J. Hyg. Camb. 66: 67-87.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

## *M.L.C.B. Agar*

***(Mannitol Lysine Crystal Violet Brilliant Green Agar)***

**LAB116**

**Description**

A medium for the selective isolation of *Salmonella* spp. (with the exception of *S. typhi* and *S. paratyphi A*) from food and faeces. *Salmonella* colonies are recognised by distinctive colonial appearance and $H_2S$ production and like the Bismuth Sulphite Agar of Wilson & Blair, this medium will detect lactose and sucrose fermenting strains. Some problems may occur with $H_2S$ negative strains, eg *S. pullorum, S. senftenberg, S. sendai* and *S. berta.* This medium should not be used to detect *S. typhi* and *S. paratyphi A,* as these strains are more susceptible to the brilliant green dye.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 5.0 |
| Tryptone | 5.0 |
| Meat Peptones | 7.0 |
| Sodium chloride | 4.0 |
| Mannitol | 3.0 |
| L-Lysine HCL | 5.0 |
| Sodium thiosulphate | 4.0 |
| Ferric ammonium citrate | 1.0 |
| Brilliant green | 0.012 |
| Crystal violet | 0.01 |
| Agar No. 2 | 15.0 |

**Method for reconstitution**

Weigh 49 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil with frequent agitation to completely dissolve the powder. Cool to 47˚C and pour plates. DO NOT AUTOCLAVE OR OVERHEAT.

**Appearance:** Pale purple, translucent gel.

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms: *Salmonella* spp. WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface plating, streaking for single colonies. Inoculation can be carried out directly, or from enrichment broths. Because of the low selectivity of this medium the inoculum should not be heavy, and it is recommended that this medium should be used in conjunction with other more selective media.

**Incubation:** 37˚C aerobically for 24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *Salmonella* spp. | 2.0-3.0 | CV.E.G. | Black |
| *Salmonella* spp. ($H_2S$ negative) | 2.0-3.0 | CV.E.G. | Pale |
| *Proteus* spp. | 1.0-2.0 | CV.E.G. | Grey brown |
| *Shigella* spp. | | | mainly inhibited |
| *Citrobacter* spp. | 0.5-2.5 | CV.E.G. | Mainly inhibited pale may have black centre |
| *E. coli* | mainly inhibited | | |
| Gram positive organisms | mainly inhibited | | |

**References**

Inove *et al.* 66th meeting of the Japanese Vet. Medicine Society.

## *ColourScreen™ MLSTB-MT (ISO)*

***Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan (ISO)***

**LAB077**

**Description**

Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan (ISO) is used for the presumptive enumeration of *Escherichia coli* from milk and milk products using the Most Probable Number (MPN) technique according to ISO 11866-1:2005.

The original Lauryl Tryptose Broth described by Mallmann and Darby (1941) has been modified to incorporate 4-Methylumbelliferyl-β-D-glucuronide (MUG) and tryptophan allowing presumptive *E. coli* to be enumerated from presumptive coliforms. The addition of MUG to the medium allows the positive discrimination of *E. coli* strains. As the majority of *E. coli* produce the β-glucuronidase enzyme, they are able to hydrolyse MUG, releasing a fluorogenic compound. Tryptophan acts as a substrate for the indole test. Tubes which fluoresce under UV are confirmed for *E. coli* by a positive indole reaction when indole (Kovac's) reagent is added to the tube. Tubes showing gas formation are identified as being positive for presumptive coliforms.

Phosphate buffers and sodium chloride improve gas production by slow lactose fermenting organisms whilst sodium lauryl sulphate acts as a selective agent for the inhibition of non-coliform organisms.

Modified Lauryl Sulphate Tryptose Broth with MUG & Tryptophan (ISO) is described in ISO 11866-1:2005 and is used in the HPA National Standard Method D5 for Enumeration of coliforms and presumptive *Escherichia coli* by the Most Probable Number (MPN) technique.

| Typical Formula | g/litre |
|---|---|
| Tryptose | 20.0 |
| Lactose | 5.0 |
| Dipotassium hydrogen phosphate | 2.75 |
| Potassium dihydrogen phosphate | 2.75 |
| Sodium chloride | 5.0 |
| Sodium lauryl sulphate | 0.1 |
| 4-Methylumbelliferyl-β-D-glucuronide (MUG) | 0.1 |
| Tryptophan | 1.0 |

**Method for reconstitution**

Disperse 36.7g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and heat gently to dissolve. Dispense 10 ml volumes into test tubes (e.g. 16mm x 160mm tubes of non-autofluorescent glass) containing inverted Durham's tubes. Sterilise at 121ºC for 15 minutes. This medium may also be used at double-strength (73.4g/l).

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid

**pH:** 6.8 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Enterococcus faecalis* WDCM 00087 (inhibition)

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.
Prepared media: 1 month at 2-8°C in the dark.

**Inoculation:** Add 1ml of test sample per tube of single-strength medium. For double-strength medium add 10ml of test sample per tube. Repeat for each further dilution.

**Incubation:** Incubate all inoculated tubes at 30°C for 24 hours (± 2 hours). Incubation may be extended for up to 48 hours (± 2 hours) if gas formation and/or turbidity not observed at 24 hours (± 2 hours).

After incubation, add 0.5ml sodium hydroxide solution to all tubes. Examine for fluorescence under a long-wave (360-366nm) UV lamp. Add 0.5ml indole (Kovac's) reagent to all tubes showing fluorescence. Mix & observe after 1 minute. The presence of indole (positive tube) is indicated by the formation of a red colour in the alcoholic phase.

**Interpretation:** Presumptive *E. coli* are indicated by those tubes showing fluorescence and a positive indole reaction.

Presumptive coliforms are indicated by those tubes which show gas formation.

Counts should then be performed according to Most Probable Number (MPN technique).

**References**

Health Protection Agency National Standard Method. Enumeration of coliforms and presumptive *Escherichia coli* by the Most Probable Number (MPN) technique. Reference number D5i2.4; issued May 2005.
ISO 11866-1:2005 Milk and milk products – Enumeration of presumptive *Escherichia coli* – Part 1: Most probable number technique using 4-methylumbelliferyl-β-D-glucuronide (MUG).

Mallmann, W.L. and Darby, C.W. (1941). *Am. J. Pub. Hlth*. **31**: 127-134.

## *Modified Giolitti and Cantoni Broth (ISO)*

**LAB219**

**Description**

Modified Giolitti and Cantoni Broth (ISO) is used for the detection and enumeration of coagulase-positive staphylococci from food and animal feeding stuffs using the Most Probable Number (MPN) technique according to ISO 6888-3:2003.

Originally described by Giolitti and Cantoni as a medium for the enrichment of staphylococci from foodstuffs, Mossel later applied the medium to use with samples from dried milk and infant food.

Optimised for use in samples where staphylococci may be stressed and/or in low numbers, growth of the target organisms is promoted by sodium pyruvate, Glycine and the high concentration of mannitol. Selectivity is achieved via lithium chloride, which inhibits Gram-negative bacilli, and potassium tellurite, which inhibits Gram-positive organisms other than staphylococci. Further selectivity is achieved by use of anaerobiosis either by pouring a plug of agar/paraffin or by incubation in a jar or incubator under anaerobic conditions. Anaerobiosis particularly inhibits the growth of *Micrococcus* spp.

The presence of coagulase-positive staphylococci is indicated by the reduction of tellurite, resulting in a blackening of the broth or a black precipitate. Coagulase-positive staphylococci are principally *Staphylococcus aureus* but may also include the species *Staphylococcus intermedius* and *Staphylococcus hyicus*.

| Typical Formula | g/litre |
|---|---|
| Peptone | 1.0 |
| Yeast extract | 5.0 |
| Lithium chloride | 5.0 |
| Mannitol | 20.0 |
| Sodium chloride | 5.0 |
| Glycine | 1.2 |
| Sodium pyruvate | 3.0 |

**Method for reconstitution**

For single-strength media: disperse 54.2g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and add 1g polyoxyethelene sorbitan mono-oleate (Polysorbate 80). Swirl to disperse and heat gently to dissolve. Dispense the medium in appropriate quantities into tubes of suitable dimensions e.g. 9ml in 16mm x 160mm tubes. Sterilise at 121ºC for 15 minutes.

For double-Strength media: disperse 108.4g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and add 2g polyoxyethelene sorbitan mono-oleate (Polysorbate 80). Swirl to disperse and heat gently to dissolve. Dispense the medium in appropriate quantities into tubes of suitable dimensions e.g. 10ml in 20mm x 200mm tubes. Sterilise at 121ºC for 15 minutes.

If product is to be used on day of preparation, allow to cool to 44-47ºC and use immediately.

If the medium is not used as above then the medium must be re-heated to 100ºC for 15 minutes to expel any dissolved oxygen and cooled to 44-47ºC.

Prior to use add X043 1% Potassium Tellurite to give a final concentration of 0.1g/L, e.g. add 0.1ml X043 to 9ml of single strength base or add 0.2ml X043 to 10ml of double strength base. DO NOT REHEAT MEDIA CONTAINING POTASSIUM TELLURITE.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw liquid (will be darker if prepared at double-strength)

**pH:** 6.9 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Staphylococcus aureus* WDCM 00034
*Escherichia coli* WDCM 00013

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: use immediately after preparation. The sterilised base medium can be stored at 2-8°C for 1 week but prior to use heat the base medium at 100ºC for 15 minutes to expel any dissolved oxygen, cool to 44-47ºC and aseptically add X043 as per directions for use.

**Inoculation:**

Detection method: add 1g/1ml of sample to 9ml/9g single-strength medium or 10g/10ml of sample to 10ml/10g double-strength broth.

Enumeration method: add 1ml of sample to each of three tubes of single-strength medium or 10ml of sample to each of three tubes of double-strength medium. Repeat for any subsequent dilutions.

**Incubation:** Incubate anaerobically (either by agar/paraffin plug in each tube or under anaerobic conditions in a gas jar or anaerobic workstation) at 37°C for 24 to 48 hours (± 2 hours).

**Interpretation:** Formation of a black precipitate or the blackening of the broth indicates the presence of coagulase-positive staphylococci.

**Sub-culture:**

24 hours: tubes suspected as positive for coagulase-positive staphylococci after 24 hours should be confirmed by sub-culture on to either Baird-Parker Medium (LAB285+X085) or Rabbit Plasma Fibrinogen Agar (LAB285+X086). Suspected positive tubes should then be reincubated for full 48 hours.

48 hours: tubes suspected as positive at 48 hours should be confirmed by sub-culture on to either Baird-Parker Medium (LAB285+X085) or Rabbit Plasma Fibrinogen Agar (LAB285+X086).

Tubes showing a presumptive negative result after 48 hours incubation should also be sub-cultured on to either Baird-Parker Medium or RPF Agar.

**References**

Giolitti, G. and Cantoni, C. (1966). A medium for the isolation of staphylococci from foodstuffs. J. Appl. Bacteriol. 29:395-398.

Mossel, D.A.A., Harrewijn, G.A. and Elzebroek, J.M. (1973). UNICEF.

ISO 6888-3:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 3: Detection & MPN technique for low numbers.

## *M.R.S. Agar*

***(de Man, Rogosa and Sharpe Agar)***

**LAB093**

**Description**

MRS Agar is a medium for the cultivation and enumeration of *Lactobacillus* spp.

Originally developed in 1960 by de Man, Rogosa & Sharpe, the medium is suitable for most lactic acid bacteria and is intended as a substitute for Tomato Juice Agar.

When acidified to pH 5.4 M.R.S. Agar can be used to enumerate *Lactobacillus bulgaricus* in yoghurts.

Nutrition is provided by a mixture of carefully selected peptones, glucose, beef & yeast extracts whilst Polysorbate 80, magnesium and manganese sulphates act as growth stimulants. Selectivity against streptococci & moulds is provided by ammonium citrate and sodium acetate. Used at low pH, ammonium citrate allows growth of lactobacilli whilst inhibiting a number of other organism groups.

Occasionally, sterilisation of this medium at 121˚C for 15 minutes, in some autoclaves, may cause the pH to fall outside of the specified pH limits 6.4 +/- 0.2. In these rare cases, adjustment of the medium using acetic acid or sodium hydroxide is recommended.

| Typical Formula | g/litre |
|---|---|
| Mixed Peptones | 10.0 |
| Yeast Extract | 5.0 |
| Beef Extract | 10.0 |
| Glucose | 20.0 |
| Dipotassium phosphate | 2.0 |
| Sodium acetate | 5.0 |
| Triammonium citrate | 2.0 |
| Magnesium sulphate | 0.2 |
| Manganese sulphate | 0.05 |
| Polysorbate 80 | 1.08 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 70 grams of powder and add 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and pour into sterile Petri dishes. If acidified medium is required, adjust pH prior to pouring. Dry the agar surface before use.

**Appearance:**
Powder: fine, slightly cohesive, light tan powder with some lumps
Finished medium: Light amber, clear gel

**pH:** 6.4 ± 0.2

**Hazard classification:** NR – Not regulated

Minimum Q.C. organisms:
*Lactobacillus casei* subsp. *rhamnosus* WDCM 00101
*Lactobacillus plantarum* ATCC 8014
*Lactobacillus delbrueckii subsp. lactis* ATCC 4797

**Storage of Prepared Medium:**

Dehydrated culture media: 10-25˚C.

Final medium: Plates - 7 days at 2-8˚C.

Capped containers – up to 1 month at 15-20˚C in the dark.

**Inoculation:** Surface, spread to cover surface, or use pour plate technique.

**Incubation:** 25˚C microaerobically for 2-5 days.

**Interpretation:** Count all colonies exhibiting typical morphology.

**References**

de Man, J.C.,. Rogosa, M and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23: 130-135.

# *MRS Agar (ISO)*

***de Man, Rogosa & Sharpe Agar at pH 5.7 according to ISO 15214:1998***

**LAB223**

**Description**

MRS Agar (ISO) is a medium for the enumeration of mesophilic lactic acid bacteria according to ISO 15214:1998.

This medium was originally developed in 1960 by de Man, Rogosa & Sharpe for the cultivation and enumeration of *Lactobacillus* spp. from various sources and is intended as a substitute for Tomato Juice Agar. This original formulation has been adapted and adjusted to pH 5.7 according to ISO 15214:1998.

Nutrition is provided by enzymatic digest of casein, glucose, meat & yeast extracts whilst polyoxyethylenesorbitan monooleate, magnesium and manganese sulphates act as growth stimulants. Selectivity against streptococci & moulds is provided by ammonium citrate and sodium acetate. Used at low pH, ammonium citrate allows growth of lactobacilli whilst inhibiting a number of other organism groups.

Although MRS Agar (ISO) is optimised to be selective for Lactobacilli, some growth of *Leuconostoc* spp. and Pediococci may also occur.

Occasionally, sterilisation of this medium at 121oC for 15 minutes, in some autoclaves, may cause the pH to fall outside of the specified pH limits 5.7 +/- 0.1. In these rare cases, adjustment of the medium using acetic acid or sodium hydroxide is recommended.

| **Typical Formula** | **g/litre** |
|---|---|
| Enzymatic digest of casein | 10.0 |
| Meat extract | 10.0 |
| Yeast extract | 4.0 |
| Triammonium citrate | 2.0 |
| Sodium acetate | 5.0 |
| Magnesium sulphate heptahydrate | 0.2 |
| Manganese sulphate tetrahydrate | 0.05 |
| Dipotassium hydrogen phosphate | 2.0 |
| Glucose | 20.0 |
| Polyoxyethylenesorbitan monooleate | 1.08 |
| Agar | 15.5 |
| **Grams per litre** | **70.0** |

**Method for reconstitution**

Disperse 70 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface before use.

**Appearance:**

Powder: fine, slightly cohesive, light tan powder with some lumps

Finished medium: clear, tan gel

**pH:** 5.7 ± 0.1

**Hazard classification**
NR – Not regulated

Minimum Q.C. organisms:
*Lactobacillus sakei subsp. sakei* WDCM 00015
*Lactococcus lactis subsp. lactis* WDCM 00016
*Escherichia coli* WDCM 00013 (inhibited)

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.
Prepared media: 7 days at 2-8°C.

**Inoculation:** Using the pour plate method, inoculate 1ml of the test sample (serial dilutions should be used) in to the Petri dish, before pouring over the molten agar. Mix carefully & allow to solidify.

Overlays may be used if required.

Surface inoculations may also be used

**Incubation:** Incubate microaerobically at 30°C for 72 hours ± 3 hours.

**Interpretation:** Count all colonies.

Some *Leuconostoc* spp. may form large, slimy colonies which may hinder the development of other colonies, thus causing an underestimation of the number of lactic acid bacteria.

Due to the possible development of microorganisms other than lactic acid bacteria, it may be necessary in some cases to confirm the colonies obtained using simple techniques such as Gram stain or test for catalase.

**References**

de Man, J.C., Rogosa, M and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol*. **23**, 130-135.

ISO 15214:1998 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony count technique at 30°C.

# *M.R.S. Broth*

***(de Man, Rogosa and Sharpe Broth)***

**LAB094**

**Description**

MRS Broth is a medium for the cultivation and enumeration of *Lactobacillus* spp. This product has the same formulation as LAB093 MRS Agar with the omission of agar.

Originally developed in 1960 by de Man, Rogosa & Sharpe, the medium can be used for confirmatory tests on organisms isolated on MRS Agar. The medium can also be used for enumeration by the Miles and Misra technique.

Nutrition is provided by a mixture of carefully selected peptones, glucose, beef & yeast extracts whilst Polysorbate 80, magnesium and manganese sulphates act as growth stimulants. Selectivity against streptococci & moulds is provided by ammonium citrate and sodium acetate.

Occasionally, sterilisation of this medium at 121˚C for 15 minutes, in some autoclaves, may cause the pH to fall outside of the specified pH limits 6.4 +/- 0.2. In these rare cases, adjustment of the medium using acetic acid or sodium hydroxide is recommended.

| Typical Formula | g/litre |
|---|---|
| Mixed Peptones | 10.0 |
| Yeast Extract | 5.0 |
| Beef Extract | 10.0 |
| Glucose | 20.0 |
| Potassium phosphate | 2.0 |
| Sodium acetate | 5.0 |
| Magnesium sulphate | 0.2 |
| Manganese sulphate | 0.05 |
| Polysorbate 80 | 1.08 |
| Ammonium citrate | 2.0 |

**Method for reconstitution**

Weigh 55 grams of powder and add 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then warm to completely dissolove solids. Dispense into suitable tubes or bottles then sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:**

Powder: fine, slightly cohesive, light tan powder with some lumps
Finished medium: Light amber, clear

**pH:** 6.4 ± 0.2

**Hazard classification:** NR – Not regulated

Minimum Q.C. organisms:
*Lactobacillus casei* subsp. *rhamnosus* WDCM 00101
*Lactobacillus plantarum* ATCC 8014
*Lactobacillus delbrueckii subsp. lactis* ATCC 4797

**Storage of Prepared Medium:**

Dehydrated culture media: 10-25°C.

Final medium: capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Either with suspect colonies from M.R.S. agar or with serial dilutions of test material.

**Incubation:** 25˚C microaerobically for 2-5 days.

**Interpretation:** For enumeration purposes count tubes showing signs of growth as positive.

**References**

de Man, J.C., Rogosa, M. and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23, 130-135.

# *MSRV*

***(Semi-solid Rappaport Medium)***

**LAB150**

**Description**

MSRV was developed in 1986 by De Smedt, Bolderdijk and Rappold as a rapid means of *Salmonella* detection. The medium, based upon Rappaport Vassiliadis broth, is inoculated directly from the pre-enrichment medium, in the centre of the plate. Motile organisms spread from the centre in the semi-solid agar, but non-salmonellas are inhibited by the selective agents.

After overnight incubation the use of polyvalent salmonella antisera or a latex kit can confirm the presence of a *Salmonella.* Alternatively, a paper disc wetted with polyvalent H antiserum can be placed 1/3 of the way from the edge of the dish, and will signal the presence of a *Salmonella* by inhibiting the mobility of the organism around the disc.

Using this medium De Smedt and Bolderdijk have reported the possibility of detecting *Salmonella* in 24hrs (1987)

| Typical Formula | g/litre |
|---|---|
| Tryptone | 2.3 |
| Meat Peptone | 2.3 |
| Acid Hydrolysed Casein | 4.7 |
| Sodium chloride | 7.3 |
| Potassium dihydrogen phosphate | 1.5 |
| Magnesium chloride | 10.9 |
| Malachite green | 0.037 |
| Agar No. 1 | 2.5 |

**Method for reconstitution**

Weigh 31.5 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and bring to the boil. Cool to 47˚C. and add 2 vials of X150 novobiocin supplement (10mg/vial). Mix well before dispensing.

**Appearance:** Turquoise/blue, clear, soft gel.

**pH:** 5.2 ± 0.2

Minimum Q.C organisms: *Salmonella typhimurium* WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of prepared medium:** Plates – up to 7 days at 4˚C.

**Inoculation:** From pre-enrichment broth (6-24hrs) adding 0.1ml to the centre of the plate.

**Incubation:** 37˚C. or 42 ± 0.5˚C. for 18-24 hours. Keep lid uppermost at all times.

**Interpretation:** A spreading growth indicates a *Salmonella* may be present, substantiated if a disc with polyvalent H antiserum has been added and is inhibiting the zone. This should be confirmed by subculturing from the edge of the mobility zone onto XLD and brilliant green agar and performing biochemical and serological tests. Direct latex agglutination may be carried out from the edge of the mobility zone.

**References**

De Smedt, J.M. and Bolderdijk, R.F. (1987): 'One Day Detection of *Salmonella* from Foods and Environmental Samples by Mobility Enrichment'. Fifth International Symposium on Rapid Methods and Automation in Microbiology and Immunology, Florence (1987). Brixia Academic Press.

De Smedt, J.M. and Bolderdijk, R.F., Rappold H. and Lautenschlaeger, D. Rapid Salmonella Detection in Foods in Mobility Enrichment on a Modified Semi-Solid Rappaport- Vassiliadis Medium. Journal of Food Protection 49 510-514. (1986).

De Smedt, J.M. and Bolderdijk, R.F. Dynamics of Salmonella Isolation with Modified Semi-Solid Rappaport-Vassiliadis Medium. Journal of Food Protection 50 658-661. (1987).

De Smedt, J.M. and Bolderdijk, R.F. Collaborative Study of the International Office of Cocoa. Chocolate and Sugar Confectionery on the Use of Mobility Enrichment for Salmonella Detection in Cocoa and Chocolate. Journal of Food Protection 53 659-664. (1990).

Goossens, H., Wauters, G., De Boeck, M., Janssens, M., and Butzler, J.P. Semi-solid selective mobility enrichment medium for isolation of Salmonella from faecal specimens J. Clin. Microbiol 19 940-941. (1984).

## *Mueller Hinton Agar*

**LAB039**

### Description

A medium for antimicrobial sensitivity testing by the disc diffusion method. This medium, used in the technique of Bauer and Kirby, has been adopted by the National Committee for Clinical Laboratory Standards (NCCLS) in the USA as the definitive method for susceptibility testing. The medium has a very low thymine and thymidine content, making it suitable for trimethoprim and sulphonamide testing, controlled to ensure correct zone sizes with aminoglycoside and tetracyline antibiotics. The medium was originally formulated as a heat labile protein free medium for the isolation of pathogenic *Neisseriaceae.*

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 2.0 |
| Acid Hydrolysed Casein | 17.5 |
| Starch | 1.5 |
| Agar No. 1 | 17.0 |
| Calcium ions | 50-100mg/litre |
| Magnesium ions | 20-35mg/litre |

### Method for reconstitution

Weigh 38 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise at 121˚C for 15 minutes. Cool to 47˚C, mix well and pour plates.

**Appearance:** Straw coloured, clear gel.

**pH:** 7.3 ± 0.1

Minimum Q.C. organisms: *E. coli* WDCM 00013
*S. aureus* (antibiotic sensitivity zones) WDCM 00034

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, inoculum as described by N.C.C.L.S.

**Incubation:** As recommended by methodology for particular organisms and antibiotics by NCCLS.

### References

Mueller, J.H. and Hinton, J. (1941). Protein-free medium for primary isolation of gonococcus and meningococcus. Proc. Soc. Exp. Biol. and Med., 48: 330-333.

Goodale, W.I., Gould, G. and Schwab, L. (1943). Laboratory Identification of sulphonamide resistant gonococcic infection. J.Am. Med. Ass., 123: 547-549.

American Public Health Association. (1950). Diagnostic Procedures and Reagents. 3rd edn., A.P.H.A., New York.

NCCLS. (1986). Performance standards for antimicrobial susceptibility testing – second informational supplement.

## *Mueller Hinton Broth*

**LAB114**

### Description

This medium is the broth version of Mueller Hinton Agar. It is an antagonist free medium for use in the tube dilution technique for the determination of antibiotic M.I.C. values. The medium is carefully standardised to meet N.C.C.L.S. standards for antimicrobial susceptibility tests on bacteria which grow aerobically.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 2.0 |
| Acid Hydrolysed Casein | 17.5 |
| Starch | 1.5 |
| Calcium ions | 50 mg/litre |
| Magnesium ions | 20 mg/litre |

### Method for reconstitution

Weigh 21 grams powder, disperse in 1 litre distilled water. Allow to soak for 10 minutes, swirl to mix then heat gently to dissolve. Distribute into tubes or bottles, and sterilise at 121˚C for 10 minutes.

**Appearance:** Pale straw, cloudy.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00034
*E. coli* WDCM 00013
(M.I.C. values)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Standard inocula are required. As described by NCCLS.

**Incubation:** As recommended by methodology for particular organisms and antibiotics by NCCLS.

### References

MacFaddin, J. (1985). Media for isolation cultivation, identification maintenance of medical bacteria. Williams & Williams, Baltimore.

N.C.C.L.S.-M7-A. (1985). Methods for dilution antimicrobiol susceptibility tests for bacteria that grow aerobically. Approved Standard.

# *Mueller-Kauffmann Tetrathionate novobiocin Broth (MKTTn)*

**LAB202**

**Description**

A selective enrichment medium for the isolation of salmonellae from food and animal feeds. The recent addition of novobiocin is to inhibit the growth of *Proteus* spp.

| Typical Formula | g/litre |
|---|---|
| Meat Extract | 4.3 |
| Enzymatic digest of casein | 8.6 |
| Sodium chloride | 2.6 |
| Calcium carbonate | 38.7 |
| Sodium thiosulphate (anhydrous)* | 30.45 |
| Ox bile | 4.78 |
| Brilliant green | 0.0096 |

*Equivalent to 47.8g of sodium thiosulphate pentahydrate.

**Method for reconstitution**

Weigh 89.4 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and bring to the boil. Cool to below 45°C, prior to use add 20 ml of iodine-iodide solution and 4 vials of X150 Novobiocin. Mix well and distribute into sterile containers.

Iodine-iodide solution

Dissolve 25g of potassium iodide in 10 ml of water. Add 20g iodine and dilute to 100ml with sterile deionised water.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: Green turbid solution that precipitates on standing

**pH:** 8.2 ± 0.2 (base medium)

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Salmonella Typhimurium* WDCM 00031
*Escherichia coli* WDCM 00013

**Storage:**

Dehydrated culture media: 10-25°C

Base medium (without supplements): 2 weeks at 10-25°C

Complete medium: use on day of preparation

**Inoculation and Incubation:** Following pre-enrichment in non-selective liquid media (see ISO 6579:2002), transfer 1ml of the broth to 10ml of MKTTn and 0.1ml to 10ml of RVS broth (LAB086). Incubate LAB202 MKTTn at 37°C ± 1°C for 24h ± 3h and LAB086 RVS broth at 41.5 ± 1°C, for 24h ± 3h. Subculture these selective broths onto XLD agar (LAB032) and a second isolation medium of your choice and incubate for 24h ± 3h. Salmonella should be confirmed by appropriate biochemical and serological techniques.

**References**

BS EN ISO 6579:2002 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

# *Nutrient Agar*

**LAB008**

**Description**

A general purpose medium for the cultivation of organisms that are not demanding in their nutritional requirements e.g. organisms that can be isolated from air, water, dust etc. Nutrient Agar is suitable for teaching and demonstration purposes, it is isotonic and can be enriched with biological fluids such as sterile blood and egg yolk.

| Typical Formula | g/litre |
|---|---|
| Peptone | 5.0 |
| Beef Extract | 3.0 |
| Sodium chloride | 8.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 28 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121˚C. Cool to 47˚C, mix well then pour plates.

**Appearance:** Buff, opalescent gel.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00034
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Surface streaking for single colonies.

**Incubation:** Temperature and time to suit organisms. Usually aerobic.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. aureus* | 1.0-2.0 | CV.E.G. | White-Yellow | |
| other *Staphylococcus* spp. | 0.5-2.0 | CV.E.G. | White-Yellow | |
| *Strep. pyogenes* | P.P.-0.5 | CV.E.G. | Transp. | |
| *E. coli* | 1.5-2.5 | CV.E.G. | Grey | |
| *Proteus* spp. | spreading | - | Grey | fishy odour |
| *Klebsiella* spp. | 2.0-4.0 | CV.E.G. | Grey | mucoid |
| *Bacillus* spp. | 2.0-6.0 | various | Grey | may spread |
| *Ps. aeruginosa* | 2.0-4.0 | F.CR.D. | Grey-Green | odour if pigmented |

## *Nutrient Broth "E"*

**LAB068**

**Description**

An inexpensive broth for the growth of nutritionally non-demanding organisms. Ideal for teaching purposes.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 1.0 |
| Yeast Extract | 2.0 |
| Peptone | 5.0 |
| Sodium chloride | 5.0 |

**Method for reconstitution**

Weigh 13 grams of powder, add to1 litre of deionised water. Heat to dissolve then dispense into bottles or tubes. Sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Straw coloured, clear.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00034
*E. coli* WDCM 00031

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation and incubation:** To suit chosen organism.

**Growth indicator:** Turbidity.

## *Nutrient Broth No. 2*

**LAB014**

**Description**

A general purpose broth which can be used for sterility testing for aerobic organisms. This broth can also be used as the suspending medium for cooked meat granules for the cultivation of anaerobic organisms.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 10.0 |
| Peptone | 10.0 |
| Sodium chloride | 5.0 |

**Method for reconstitution**

Weigh 25 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then dispense into tubes or bottles, and sterilise for 15 minutes at 121˚C.

**Appearance:** Pale straw, clear.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00034
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Incubation:** 37˚C for 18-24 hours aerobically.

**References**

British Pharmacopoeia. (1973). H.M.S.O., London. Cruikshank, R. (1972). Medical Microbiology. 11th edn. Livingstone, London.

## *O157 Broth MTSB*

***(Modified Tryptone Soy Broth)***

**LAB165**

**Description**

Modified tryptone soy broth has emerged as the medium of choice for the enrichment of *E. coli* O157:H7 in red meats[1,2]. As concern regarding this organism has grown due to the severity of the disease syndromes caused, and the increase in foodborne infection[3], so too has the need to optimise methods for its efficient isolation. Symptoms start with severe stomach cramps and watery, bloody diarrhoea, and a percentage of individuals infected will develop Haemolytic Uraemic Syndrome (HUS) leading to acute renal failure[4]. In a comparison of 4 different selective broth media, MTSB was the most productive and selective for the isolation of *E. coli* O157:H7. MTSB is made selective for O157:H7 by including bile salts in the dehydrated medium, and the addition of novobiocin supplement (X150).

| Typical Formula | g/litre |
|---|---|
| Tryptone | 17.0 |
| Sodium chloride | 5.0 |
| $K_2HPO_4$ | 4.0 |
| Dextrose | 2.5 |
| Soy Peptone | 3.0 |
| Bile Salts No.3 | 1.5 |

**Method for reconstitution**

Weigh 33 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and autoclave at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of Novobiocin supplement X150. Mix well and distribute aseptically into sterile containers.

**Appearance:** Clear straw broth

**pH:** 7.2 ± 0.2

Minimum QC organisms: *E. coli* O157:H7 (non-toxigenic) WDCM 00014
*E. coli* WDCM 00013 (inhibition)

**Inoculation:** Add 25g sample to 225ml of supplemented MTSB and homogenise for 2 minutes.

**Incubation:** 42˚C aerobically for 24hrs. Subculture onto CT-SMAC (LAB161 plus X161) or SMAC-BCIG (HA006) and examine for non-sorbitol fermenting colonies and/or glucuronidase negative organisms. Some workers[1] recommend the use of an immunomagnetic separation step after 6hrs incubation.

**Interpretation:** Turbidity in the broth indicates growth. All broths should be subcultured to selective media whether turbid or not.

**References**

1) Bolton, E.J., Crozier, L., Williamson, J.K. (1995) Optimisation of methods for the isolation of *Escherichia coli* O157 from beefburgers. PHLS Microbiology Digest 12 (2) 67-70.

2) Willshaw, G.A., Smith, H.R., Roberts, D., Thirlwell, J., Cheasty, T., Rowe, B. (1993) Examination of raw beef products for the presence of verocytotoxin producing *Escherichia coli,* particularly those of serogroup O157. J.Appl.Bacteriol. 75 420-426.

3) Sharp, J.C.M., Coia, J.E., Curnow, J., Reilly, W.J. (1994) *Escherichia coli* O157 infections in Scotland. J.Med.Microbiol 40 3-9.

4) Doyle, M.P. (1991) *Escherichia coli* O157:H7 and its significancein foods. Int.J.Food Microbiol. 12 289-302.

# *Orange Serum Agar*

**LAB147**

**Description**
A medium developed for the investigation of organisms involved in the spoilage of citrus products including fruit juices and citrus concentrates. The low pH of these products restricts the growth of organisms to those capable of tolerating an acid environment such as yeasts and moulds and bacteria belonging to the genera *Bacillus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Clostridium*. By having a low pH and incorporating orange extract, Orange Serum Agar is the ideal isolation medium.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Yeast Extract | 3.0 |
| Orange Extract | 5.0 |
| Glucose | 4.0 |
| Di-potassium phosphate | 3.0 |
| Agar No.2 | 17.0 |

**Method for reconstitution**
Weigh 42 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes. Bring to the boil, swirling frequently. Sterilise by autoclaving for 15 minutes at 115°C. Cool to 47°C, mix well and dispense into Petri dishes.

**Appearance:**

**Powder:** fine, free-flowing, homogeneous, buff

**Finished medium:** Amber, slightly opalescent gel

**pH:** 5.5 ± 0.2

Minimum Q.C. organisms *Lactobacillus plantarum*
*Aspergillus niger*

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C. in the dark. Capped containers – 10-25°C. in the dark.

**Inoculation:** Pour plate technique.

**Incubation:** 3 days at 30°C. for bacteria, 5 days at 30˚C for yeasts and moulds.

**Interpretation:** Count bacterial colonies and yeasts/moulds separately. Calculate the colony forming units (CFU) per ml of the sample, allowing for dilution factors.

**References**

Hays, G.L. (1951) The isolation, cultivation and identification of organisms which have caused spoilage in frozen concentrated orange juice. Proc. Florida State Hort. Soc.

Hays, G.L. and Reister, D.W. (1952) The control of 'off-odour' spoilage in frozen concentrated orange juice. Food Tech 6 p386.

Murdock, D.I., Folinazzo, J.F., and Troy, V.S. (1952) Evaluation of plating media for citrus concentrates. Food Tech. 6 p181.

# *ORSIM*

***(Oxacillin Resistant Staphylococci Isolation Medium)***

**LAB192**

**Description**
The over-prescription of therapeutic antibiotics in recent years is thought to be a contributing factor in the rising numbers of multi-resistant bacteria being encountered. One such bacterium, MRSA (multi resistant *Staphylococcus aureus*), is particularly prevalent within the hospital environment, and is well recognised as a pathogen amongst immuno-compromised patients. In this situation, early detection is vital to ensure the individual's survival. This media is an improved version of the highly regarded Mannitol Salt Agar (LAB007) and incorporates an enhanced indicator system using aniline blue and mannitol fermentation. This combination produces intense blue colonies as presumptive MRSA, which are unmistakable amongst mixed cultures and easily visualised against the media background. To complement this, ORSIM possesses a refined selectivity, derived from a reduction in the salt level to 55g/L, and the introduction of Lithium chloride at 5g/L. This chemical mixture still provides the required inhibition towards competing organisms, whilst ensuring optimal recovery of MRSA, even at low numbers. To complete the medium, the selective supplement X192 is included. This contains two antibiotics, oxacillin to inhibit multi sensitive *Staphylococcus aureus* (the cause of false positives), and polymyxin B to suppress other halophillic bacteria such as *Proteus* spp.

| Typical Formula | g/litre |
|---|---|
| Peptone | 11.8 |
| Yeast Extract | 9.0 |
| Mannitol | 10.0 |
| Sodium chloride | 55.0 |
| Lithium chloride | 5.0 |
| Aniline blue | 0.2 |
| Agar | 12.5 |

**Method for Reconstitution**
Weigh 103.5 grams of powder and disperse into 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix then sterilise at 121°C for 15 minutes. Cool to 47°C, and aseptically add 2 vials of X192 supplement. Mix well, and pour into sterile Petri dishes.

**Appearance:** Straw/grey gel.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* (MRSA Strain) NCTC 11940
*E.coli* NCTC 25922 (inhibition).

**Storage of Prepared Medium:** Plates can be stored up to 7 days at 2-8°C in the dark.

**Inoculation:** Take a swab sample from a suspected infection and apply the swab end directly to the surface of a supplemented plate of ORSIM and streak out for single colonies.

**Incubation:** Aerobically at 37°C for 24 and 48 hours.

**Interpretation:** After incubation for 24 hours, examine the plate for intense blue colonies and confirm using either coagulase/latex agglutination and Penicillin binding protein 2' test (PBP2'). Once confirmed, all positive plates should be discarded safely.
*Typical strains of MRSA will be detected within 24 hours on this medium. However, some strains may require longer incubation, so all negative plates should be re-incubated for a further 24 hours*.

**References**

Orth, D.S. (1993) Handbook of Cosmetic Microbiology. Marcel Dekker, Inc., New York, NY.

Brummer, B. (1976). Influence of possible disinfectant transfer on Staphylococcus aureus plate counts after contact sampling. App. Environ. Microbiol. 32:80-84.

# *Oxytetracycline Glucose Yeast Extract Agar Base*

***(O.G.Y.E.)***

**LAB089**

**Description**

A selective medium for the enumeration of yeasts and moulds in food, introduced by Mossel in 1970. Unlike many selective media for yeasts OGYE has a neutral pH and it has been shown to give better recovery rates than those media with a low pH. Oxytetracycline is used to inhibit bacteria, certain high protein foods may reduce the effectiveness of this antibiotic as a selective agent. Rose Bengal Chloramphenicol Agar (LAB036) is recommended in these instances.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 5.0 |
| Dextrose | 20.0 |
| Biotin | 0.001 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 37 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 115°C for 10 minutes. Cool to 47°C and aseptically add 2 vials of X089 Oxytetracycline selective supplement. Mix thoroughly and pour into Petri dishes. DO NOT REHEAT THIS MEDIA ONCE PREPARED.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: Clear, straw gel

**pH:** 7.0 ± 0.2

**Hazard classification:** NR – Not regulated

Minimum Q.C. organisms: *Aspergillus* sp.
*Saccharomyces cerevisiae* WDCM 00058

**Storage of Prepared Medium:** Dehydrated culture media: 10-25oC
Prepared media: Plates - up to 7 days at 2-8oC, in the dark
Capped containers – up to 1 month at 2-8oC, in the dark

**Inoculation:** Surface spreading or pour plate.

**Incubation:** 25˚C aerobically for 5 days.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *Candida* spp. | 3.0-4.0 | CV.E.D. | Cream |
| *Candida krusei* | 7.0-8.0 | F.CR.D. | White |
| *S. cerevisiae* | 3.0-4.0 | CV.E.D. | White |
| *Pen. notatum* | 1.5 | | Green/blue centre |
| *Pen. flavescens* | 1.5-2.0 | | Yellow centre |

**References**

Mossel, D.A.A. *et al*. (1970). O.G.Y.E. for Selective Enumeration of Moulds and Yeast in Foods and Clinical Material. J. Appl. Bact. 35: 454-457.

Banks, J.G., Board, R.G. (1987). Some factors influencing the recovery of yeasts and moulds from chilled foods. Int. J. Food Microbiol. 4: 197-206.

# *PALCAM Agar Base*

***(Polymyxin, Acriflavine, Lithium chloride, Ceftazidine, Aesculin, Mannitol)***

**LAB148**

**Description**

Palcam Agar was developed by Van Netten *et al* in 1989 as an improved selective differential medium for the isolation of *Listeria monocytogenes* from food, clinical and environmental specimens.

Improved selectivity is achieved by the combination of antibiotic supplements and microaerobic incubation, whilst the double indicator system of aesculin hydrolysis and mannitol fermentation aids differentiation of *Listeria* spp from enterococci and staphylococci which can be confused with *Listeria* spp on other types of culture media.

| Typical Formula | g/litre |
|---|---|
| Columbia Peptone Mix | 23.0 |
| Sodium chloride | 5.0 |
| Corn Starch | 1.0 |
| Yeast Extract | 3.0 |
| Glucose | 0.5 |
| Mannitol | 10.0 |
| Aesculin | 0.8 |
| Lithium chloride | 15.0 |
| Ferric ammonium citrate | 0.5 |
| Phenol red | 0.08 |
| Agar No. 2 | 12.0 |

**Method for Reconstitution**

Weigh 71 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of P.A.C. Supplement – X144. Mix thoroughly and pour into Petri dishes.

**Appearance:** Red, translucent

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms *L. monocytogenes* WDCM 00020
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 4˚C in the dark.

**Inoculation:** 0.1ml of sample selectively enriched in Palcam Broth (or other enrichment medium) spread over surface of plate.

**Incubation:** 30˚C aerobically or microaerobically for 24-48 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *L. monocytogenes* | 1.5-2.0mm | F.E.D. | Grey/green | Black halo, (draughtsman colonies). |
| Other *Listeria* spp. | 0.5-2.0mm | F.E.D. | Grey/green | Black halo, (draughtsman colonies). |
| Enterococci | Inhibited | - | - | (Small yellow colonies with yellow/ green halo). |
| Staphylococci | Inhibited | - | - | (Small white/ yellow colonies with yellow/ green halo) |
| *Bacillus* spp. | Inhibited | - | - | - |

**References**

Van Netten, P., Perales, I., Curtis, G.D.W., Mossel, D.A.A. (1989) Liquid and solid selective differential media for the enumeration of *L. monocytogenes* Int. J. Food Micro. 8 (4) 299-316.

# *PALCAM Broth*

***L-PALCAM Broth (Liquid, Polymyxin, Acriflavine, Lithium chloride, Ceftazidime, Aesculin, Mannitol)***

**LAB144**

### Description

Developed by Van Netten *et al* (1989) L-Palcam is a selective differential medium for the enrichment of *Listeria* spp. in food, environmental and clinical samples. It is unique amongst Listeria enrichment media in that it contains an indicator system (aesculin) which will signal the presence of a possible *Listeria* by a browning/ blackening of the broth; the result being the indication of a potential problem up to 48 hours before growth on plating media can be observed.

| Typical Formula | g/litre |
|---|---|
| Columbia Peptone Mix | 23.0 |
| Yeast Extract | 5.0 |
| Peptonised Milk | 5.0 |
| Sodium chloride | 5.0 |
| Mannitol | 5.0 |
| Aesculin | 0.8 |
| Ferric ammonium citrate | 0.5 |
| Phenol red | 0.08 |
| Lithium chloride | 10.0 |

### Method for Reconstitution

Weigh 54.4 grams of powder and disperse in 1 litre of deionised water. Soak for 10 minutes, swirl to mix and autoclave at 121˚C for 15 minutes. Cool to 47˚C and add two vials of X144. Mix well and dispense into sterile tubes or bottles.

**Appearance:** Clear red broth

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*L. monocytogenes* WDCM 00020
*E. coli* (inhibition) WDCM 00013

**Storage of prepared medium:** Capped containers – up to 7 days at 4˚C.

**Inoculation:** Sample or pre-enriched sample added to the broth in the ratio 1 : 10.

**Incubation:** 30˚C for 24 hours and 48 hours.

**Subculture:** Onto Palcam Agar – LAB148. If low numbers of Listeria are present the medium may not produce the brown black colour. All tubes should be subcultured onto selective agar before a sample is scored as negative.

### References

Van Netten, P., Perales, I., Curtis, G.D.W., Mossel, D.A.A. (1989) Liquid and solid selective differential media for the enumeration of *L. monocytogenes* Int. J. Food Micro. 8 (4) 299-316.

# *PEMBA*

***(Bacillus Cereus Medium)***

**LAB193**

### Description

This medium is based on the highly specific and sensitive PEMBA medium. It is used for the isolation and enumeration of *Bacillus cereus*. This formulation specifically enhances egg yolk precipitation and sporulation of *Bacillus cereus*. The bromothymol blue pH indicator gives clear visualisation of alkaline mannitol non-fermenting colonies and egg yolk precipitation indicative of *B. cereus*. The selectivity is provided by the polymyxin B supplement (X193) and provides excellent results for the majority of sample types.

Microscopic examination of presumptive *B. cereus* colony can confirm identity by presence of lipid globules in vegetative cells.

| Typical Formula | g/litre |
|---|---|
| Peptone | 1.0 |
| Mannitol | 10.0 |
| Sodium chloride | 2.0 |
| Magnesium sulphate | 0.1 |
| Disodium hydrogen phosphate | 2.5 |
| Bromothymol blue | 0.12 |
| Sodium pyruvate | 10.0 |
| Agar | 15.0 |

### Method for Reconstitution

Weigh 41g of powder and disperse in 950ml of deionised water, allow the mixture to soak for 10 minutes, swirl to mix and sterilize by autoclaving for 15 minutes at 121ºC for 15 minutes. Cool to 47ºC and add two vials of X193 and 50ml of Egg Yolk Emulsion (X073) mix well and pour the plates. Dry the agar surface before inoculation.

**Appearance:** Yellow and opaque.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Bacillus cereus* WDCM 00001.
*Escherichia coli* WDCM 00013 (inhibition).

**Storage of Prepared Medium:** up to 7 days at 2-8ºC in the dark.

**Inoculation:** Surface spreading or streaking for single colonies.

**Incubation:** 30ºC aerobically for 24-48 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *B. cereus* | 3.0-4.0 | F.CR.D | Blue white halo |
| *B. subtilis* | 2.0-3.0 | F.CR.D | Yellow |
| *B. licheniformis* | 2.0 | F.CR.D | Yellow |
| *E. coli* | no growth | - | |
| *S. aueus* | 1.0 | CV.E.G. | Yellow white halo |

### References

Holbrook, R. & Anderson, J.M. (1980). Can. J. Microbiol., 26(7) 753-759.

Donovan, K.O. (1958). J. Appl. Bacteriol., 21(1) 100-103.

Mossel, D.A.A., Koopman, M.J. & Jongerius. E. (1967). J. Appl. Bacteriol. 15(3) 650-653.

## *Peptone Water*

**LAB104**

**Description**

A general purpose growth medium that can be used as a base for carbohydrate fermentation studies. The medium has a high level of tryptone making it suitable for use in the indole test.

| Typical Formula | g/litre |
|---|---|
| Peptone | 5.0 |
| Tryptone | 5.0 |
| Sodium chloride | 5.0 |

**Method for reconstitution**

Weigh 15 grams of powder, and disperse in 1 litre of deionised water. Allow to dissolve then distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes. If sterile additions are to be made to this medium e.g. carbohydrates, the volume of water for reconstitution must be reduced accordingly. A pH indicator may be added to detect acid production from carbohydrate utilisation.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, colourless

**pH:** 7.2 ± 0.2

**Hazard classification:** NR – Not regulated

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013 (indole positive)
*Salmonella typhimurium* WDCM 00031 (indole negative)

**Storage of Prepared Medium:**

Dehydrated culture media: 10-25°C.

Final medium: capped containers – up to 3 months at 15-20°C in the dark..

**Inoculation:** A light inoculum from a pure culture.

**Incubation:** According to organism.

**References**

Bergey's Manual of Systematic Bacteriology, Vol. 1, (1984). Williams and Wilkins, Baltimore/London.

MacFadden, J.F. (1983). Biochemical Tests for the Identification of Medical Bacteria, 2nd edn. Williams and Wilkins, Baltimore/London.

## *Perfringens Agar OPSP*

**LAB109**

**Description**

Oleandomycin, Polymixin, Sulphadiazine, Perfringens (OPSP) agar, has been used as a standard medium for Clostridium perfringens for many years. This medium was developed by Handford in 1974 to overcome some of the problems associated with enumerating Clostridium perfringens in foods. The medium is buffered and utilises sodium metabisulphite and liver extract as sources of H2S with ferric ammonium citrate as the indicator.

The medium is made selective with the addition of X109 Sulphadiazine and X110 Oleandomycin / Polymyxin supplements. Some strains of C. perfringens may demonstrate sensitivity to the sulphadiazine antibiotic (X109) in such cases use of LAB194 TSC Perfringens Agar Base (with X194 D-Cycloserine) should be considered.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 15.0 |
| Yeast Extract | 5.0 |
| Soy Peptone | 5.0 |
| Liver Extract | 7.0 |
| Ferric ammonium citrate | 1.0 |
| Sodium metabisulphite | 1.0 |
| Tris buffer | 1.5 |
| Agar No. 2 | 10.0 |

**Method for reconstitution**

Weigh 45.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Allow to cool to 47°C before adding 2 vials each of selective supplements X109 and X110. Mix well before dispensing into Petri dishes.

**Appearance:**

**Powder:** fine, free-flowing, homogeneous, buff

**Finished medium:** pale straw, clear gel

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *C. perfringens* WDCM 00007
*E. coli* WDCM 00013
(inhibition)

**Storage of prepared medium:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: Use on day of preparation.

**Inoculation:** Pour plates, 1ml sample plus 9ml medium. When set overlay with sterile medium.

**Incubation:** Incubate at 37°C anaerobically for 24 hours.

**Interpretation:** Count large black colonies, presumptively identified as *C. perfringens.*

**References**

Bowen AB, Braden CR (2006). "Invasive Enterobacter sakazakii disease in infants". Emerging Infect Dis 12 (8): 1185–9.

Handford, P.M. (1974). A new medium for the detection and enumeration of C. perfringens in foods. J. Appl. Bact. 37: 559-570.

Hauschild A.H.W. and Hilsheimer R. (1973). Evaluation and Modifications of Media for Enumeration of Clostridium perfringens. Applied Microbiology 27 p78-82.

Shahidi, S.A. and Ferguson, A.R. (1971). A new quantitative and confirmatory medium for C. perfringens in food. Appl. Microbiol. 21:500-506.

Marshall, R.S., Steenberger, J.F. and McClung, L.S. (1965). A rapid technique for the enumeration of C. perfringens. Appl. Microbiol. 13: 559.

Pharmacopoeia of culture media for food microbiology. (1987). Int. J. Food Microbiol. 5:3:240-241.

## *Perfringens Agar TSC*

***(Tryptose Sulphite Cycloserine (TSC) Agar)***

**LAB194**

**Description**

Perfringens Agar Base is a nutrient medium to which egg yolk emulsion (X073) and cycloserine (X194) are added for the preparation of Tryptose Sulphite Cycloserine (TSC) Agar. Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction by *Clostridium perfringens*. The reduction of sulphite by *Cl. perfringens* produces black colonies and the egg yolk emulsion incorporated into the media detects the lecithinase activity of this bacteria. However not all strains produce lecithinase and therefore black lecithinase positive and black lecithinase negative colonies should be considered as presumptive *Cl. perfringens*.

| Typical Formula | g/litre |
|---|---|
| Tryptose | 15.0 |
| Soy Peptone | 5.0 |
| Beef extract | 5.0 |
| Yeast extract | 5.0 |
| Sodium metabisulphite | 1.0 |
| Ferric ammonium citrate | 1.0 |
| Agar | 14.0 |

**Method for reconstitution**

Weigh 46.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 10 minutes. Allow the medium to cool to 47°C and supplement with 2 vials of X194 (cycloserine) and 50ml of egg yolk emulsion (X073), mix well and pour into sterile Petri dishes. The egg yolk emulsion is omitted for preparation of Egg Yolk Free TSC Agar and Egg Yolk Free TSC Agar should be used for an overlay medium.

**Appearance:** Straw, clear gel or pale yellow opaque gel.

**pH:** 7.6 ± 0.2

**Storage of Prepared Medium:** Plates can be stored up to 7 days at 2-8°C in the dark.

Minimum Q.C. organisms:
*Clostridium perfringens* WDCM 00007
*Escherichia coli* WDCM 00013 (inhibition)

**Inoculation:** For a spread plate inoculate the agar plate with 0.1ml aliquots of an appropriate serial dilution of the homogenised test sample and overlay if required. For a pour plate mix 1ml aliquots of an appropriate serial dilution of the homogenised test sample with approximately 20 ml of TSC plus egg yolk emulsion. For full details refer to appropriate references and standard method protocols.

**Incubation:** 35°C ± 2°C anaerobically for 18-24 hours.

**Interpretation:** Count all black colonies with or without a halo as presumptive *C. perfringens*. Further confirmation should be carried out according to standard method protocols e.g. nitrate reduction, lactose fermentation, gelatin liquefaction and absence of motility.

**References**


Shahidi, S.A. and Furguson, A.R. (1971). Appl. Microbiol. 21. 500-506.

Harmon, S.M., Kauttar, D.A. and Peeler, J.T. (1971). Appl. Microbiol. 22. 688-692.

Hauschild, A. H. W. and Hilsheimar R. (1973). Appl. Microbiol. 27. 78-82.

Hauschild A.H.W. and Hilsheimar, R. (1973). Appl. Microbiol. 27. 521-526.

Hauschild, A.H.W. *et al* (1977). Can. J. Microbiol. 23. 884-892.

Labbe, R. G. and Harmon, S.M. (1992). Compendium of methods for the microbiological examination of foods, 3rd ed 623-635. American Public Health Association, Washington, D.C.

Rhodehamel, E.J. and Harmon, S.M. (1995). Bacteriological Analytical Manual 8th ed. 16.01-16.06 AOAC International, Gaithersberg, MD.

Andrews, W. (1995) Official methods of analysis AOAC International 16th ed. 1-119. AOAC International, Arlington, VA.


## *Plate Count Agar*

**LAB149**

**Description**

A medium designed for use with the spiral plating system and other surface inoculation techniques. The formula is suitable for the determination of total viable counts in food products by surface count and pour plate methods.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 5.0 |
| Yeast Extract | 2.5 |
| Glucose | 1.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 20.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C then pour into Petri dishes.

**Appearance:** Pale straw colour, clear.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00032
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, or pour plate.

**Incubation:** 30˚C aerobically for 48 hours for aerobic mesotroph count. 6˚C aerobically for 10 days for aerobic psychrotroph count. 55˚C aerobically for 48 hours for aerobic thermotroph count.

**Interpretation:** Count all colonies or use spiral plating colony count equipment.

**References**


Reasoner, D.J., Geldreich, E.E. (1985) A New Medium for the Enumeration and Subculture of Bacteria from potable water. App & Env. Microbiol. Jan. 1985 p.1-7.

American Public Health Association (1985) Standard Methods for the Enumeration of Water and Wastewater. 16th Edition. American Public Health Association Inc. Washington D.C.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.


## *Plate Count Agar (A.P.H.A.)*

***(Standard Methods Agar, Tryptone Glucose Yeast Agar)***

**LAB010**

**Description**

Formulated to A.P.H.A. specifications, this medium is used for establishing total viable counts for aerobes in food, dairy and water bacteriology. The product uses agar of very high gel strength in order that it can be used in pour-plate as well as surface inoculation techniques. The product can be remelted prior to use although it should not be held for a prolonged period in the molten stage.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 5.0 |
| Yeast Extract | 2.5 |
| Glucose | 1.0 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 23.5 grams of powder, disperse in 1 litre of deionised water. Bring to the boil with frequent stirring to dissolve. Dispense into tubes and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 44-46˚C for not more than 3 hours prior to use.

ROLL-TUBES. Add an additional 10g/litre Agar No. 1 prior to reconstitution of the medium.

**Appearance:** Pale straw coloured, clear gel.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00032
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation method:** Pour plate technique or surface inoculation.

**Incubation:** 30˚C aerobically for 48 hours for aerobic mesotroph count. 6˚C aerobically for 10 days for aerobic psychrotroph count. 55˚C aerobically for 48 hours for aerobic thermotroph count.

**Interpretation:** Count all colonies and calculate the number of organisms (or 'colony forming units' c.f.u.) per ml of sample allowing for dilution factors.

**References**


American Public Health Association (1972). Standard Methods for the Examination of Dairy Products. 13th edn. (ed. Hausler, W.J.) A.P.H.A., Washington.

American Public Health Association (1966). Recommended Methods for the Microbiological Examination of Foods, 2nd edn. (ed. Sharf, J.M.) A.P.H.A., Washington.

American Public Health Association (1976). Standard Methods for the Examination of Water and Waste Water, 14th edn. (ed.. Franson, M.A) A.P.H.A., Washington.

# *Potato Dextrose Agar*

**LAB098**

### Description

Potato Dextrose Agar is recommended by the American Public Health Association for the enumeration of yeasts and moulds in examination of dairy products, soft drinks, dried and frozen foods and other types of product. Depending on whether the medium is to be used as a selective or non-selective agar it can be used with or without acidification.

| Typical Formula | g/litre |
|---|---|
| Potato Extract | 4.0 |
| Dextrose | 20.0 |
| Agar No. 1 | 15.0 |

### Method for reconstitution

Weigh 39 grams of powder, disperse in 1 litre of deionised water, then sterilise at 121˚C for 15 minutes. Mix well before pouring into sterile Petri dishes. In certain cases it may be desirable to lower the pH of the medium to 3.5 in order to suppress bacterial growth. This can be done by adding 10ml of sterile 10% Lactic Acid X037, to one litre of Potato Dextrose Agar LAB098. This addition must be after autoclaving and cooling to 47˚C. Once the pH has been lowered the medium may not be heated again without resultant loss of gel strength caused by agar hydrolysis.

**Appearance:** Translucent white agar.

**pH:** 5.6 ± 0.2 (3.5-4.0 if X037 is added)

Minimum Q.C. organisms:
*Aspergillus* brasiliensis WDCM 00053
*Saccharomyces cerevisiae* WDCM 00058

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 1 month at 15-20˚C in the dark.

**Inoculum:** Pour plate technique.

**Incubation:** 21˚C aerobically for 5 days.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *Candida* spp. | 2.0 | C.V.E.D. | White |
| *Candida krusei* | 2.0 | F.Rz.D. | Grey/White |
| *Asp. niger* | 4.0 | Black spores centre White surround | Yellow obverse |
| *Pen. notatum* | 4.0 | Green spores centre White surround | Green obverse |

### References


Association of Official Analytical Chemists (AOAC). Bacteriological Analytical Manual, 5th ed. (1978). Washington D.C. Hausler, W.J. (ed.).

Standard Methods for the Examination of Dairy Prod. 14th edn., Washington D.C.: American Public Health Association, (1976).


# *Pseudomonas Agar Base*

***(C.F.C./C.N. Agar)***

**LAB108**

### Description

The base medium is a modification of King's medium A which uses magnesium and potassium salts to enhance production of the pigments pyocyanin (green) and fluorescein (detected by U.V./blue light). The medium is made selective for *Pseudomonas aeruginosa* by the addition of X107 C.N. supplement. Alternatively the medium can be made selective for *Pseudomonas* species generally by the addition of X108 C.F.C. supplement. This medium can be made selective for the isolation of *Burkholderia cepacia* by the addition of X140.

| Typical Formula | g/litre |
|---|---|
| Acid Hydrolysed Casein | 10.0 |
| Gelatin Peptone | 16.0 |
| Potassium sulphate | 10.0 |
| Magnesium chloride | 1.4 |
| Agar No. 2 | 11.0 |

### Method for reconstitution

Weigh 48.4 grams of powder and disperse in 1 litre of deionised water. Add 10ml of glycerol. Sterilise by autoclaving at 121˚C for 15 minutes. Allow the medium to cool to 47˚C then add the contents of 2 vials of either X107 C.N. supplement or X108 C.F.C. supplement. Mix well and pour into Petri dishes.

**Appearance:** Pale straw, opaque.

**pH:** 7.1 ± 0.2

Minimum Q.C. organisms: *P. aeruginosa* WDCM 00025
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, spread 0.1 to 0.5ml of sample over entire surface.

**Incubation:** 25-30˚C aerobically for 48 hours.

**Interpretation:** Count all colonies as *Pseudomonas species.* Colonies that exhibit the pyocyanin and fluorescein pigments count as *P. aeruginosa.*

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | fluorescence |
|---|---|---|---|---|
| *Ps. aeruginosa* | 2.0-3.0 | CV.Cr.D. | Green/Blue | yes |
| *P. fluorescens* | 2.0-3.0 | CV.Cr.D. | Yellow | yes |
| *P. fragi* | 1.0-3.0 | CV.Cr.D. | Grey | no |

### References


Burton, M.O., Campbell, J.J.R. and Eagles, B.A. (1948). The mineral requirement for pyocyanin production. Can. J. res. Sect. C. Bot. Sci. 26:15.

King, E.O., Ward, M.K. and Raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301.

Goto, S. and Enomoto, S. (1970). Jap. J. Microbiol. 14: 65-72.

Mead, G.C. and Adams, B.W. (1977). Br. Poult. Sci. 18: 661-667.

## *R2A Broth*

**LAB203**

**Description**

A medium of low nutritional content for use with membrane methods for the enumeration of bacteria from water samples.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 0.5 |
| Meat Peptone | 0.5 |
| Casamino acids | 0.5 |
| Glucose | 0.5 |
| Starch | 0.5 |
| Dipotassium hydrogen Phosphate | 0.3 |
| Magnesium sulphate | 0.05 |
| Sodium pyruvate | 0.3 |

**pH:** 7.2 ± 0.2

**Hazard classification:** NR – Not regulated

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, pale straw liquid

**Method for reconstitution**

Disperse 3g of powder in 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix and dispense into tubes or bottles. Sterilise by autoclaving at 121ºC for 15 minutes.

Minimum QC organisms: *Aeromonas hydrophila* WDCM 00063
*Pseudomonas fluorescens* WDCM 00115
*Escherichia coli* WDCM 00013

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 1 month at 2-8°C in the dark.

## *R2A Medium*

**LAB163**

**Description**

R2A medium was developed to determine the bacterial count in potable waters during treatment and distribution, and has been shown to give significantly higher counts than plate count agar (PCA) or similar high-nutrient media. The standard plate count (SPC) method using PCA provides an enumeration of bacteria which grow best at, or near, body temperature and this estimation at best may correlate to the coliforms present in the sample. However, there will be a population of heterotrophic bacteria which cannot grow at all under the conditions of the SPC method or may grow so slowly that the colonies fail to reach a size detectable to the eye in the 48-h incubation period. In order to enumerate this section of the bacterial population in water, a medium of low nutritional content (R2A) and extended incubation times are required.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 0.5 |
| Meat Peptone | 0.5 |
| Casamino acids | 0.5 |
| Glucose | 0.5 |
| Starch | 0.5 |
| Dipotassium hydrogen Phosphate | 0.3 |
| Magnesium sulphate | 0.05 |
| Sodium pyruvate | 0.3 |
| Agar No.2 | 15.0 |

**pH:** 7.2 ± 0.2

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, opalescent gel

**Method for reconstitution**

Weigh 18 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121ºC. (If required, bring to the boil to dissolve the agar, and pour into smaller volumes before sterilizing.) Cool to 44-46°C for not more than 3 hours before use. Mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

**Inoculation:** Pour 15ml into a Petri dish containing 1ml of sample, mix well and allow to set. Pour a further 10ml as an overlay and again allow to set. Alternatively it may be used as a spread plate, inoculating 0.1ml onto the plate and spreading over the entire surface of the medium. It can also be used with membrane filters if required.

**Incubation:** When plates have set, incubate at 22°C for 5-7 days or 30°C for 3 days. Other incubation temperatures between 20°C and 28°C may be used.

**Interpretation:** Count all colonies and report the number of bacteria in the original sample as the heterotrophic plate count.

Minimum QC organisms: *Pseudomonas fluorescens* WDCM 00115
*Aeromonas hydrophila* WDCM 00063

**Storage:**

Dehydrated culture media: 10-25ºC away from direct sunlight.

Prepared media:

Plates - 7 days at 2-8°C in the dark

Capped containers – 3 months at 15-20°C in the dark

**References**

Reasoner, D.J., Geldreich, E.E. (1985) A New Medium for the Enumeration and Subculture of Bacteria from potable water. App & Env. Microbiol. Jan. 1985 p. 1-7.

American Public Health Association (1985) Standard Methods for the Enumeration of Water and Wastewater. 16th Edition. American Public Health Association Inc. Washington DC.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

## *Raka-Ray No.3 Agar*

**LAB198**

**Description**

Based on the formulation of Saha, Sondag and Middlekauff, Raka-Ray No.3 Agar is for the detection of lactic acid bacteria in beer and for monitoring in-process beer quality. It is recommended for this application by the European Brewing Convention (EBC) and the American Society of Brewing Chemists (ASBC).

Contamination of beer and the beer making process by members of the lactobacilli family results in spoilage, primarily through their production of metabolic products which are detrimental to the flavour of the final product. Detection of these organisms is complicated by their diverse nutritional and environmental requirements.

A number of different formulations have been described for the isolation of lactic acid bacteria in brewing products and processes. Raka-Ray Agar was developed by the addition of various growth promoting compounds to Universal Beer Agar. This work led to the recognition that the addition of sorbitan mono-oleate, liver extract and N-acetylglucosamine produced superior growth when compared to the standard Universal Beer Agar formulation.

Further investigations provided the basis for the final formula of Raka-Ray No. 3 Medium in which fructose is an essential carbohydrate source for *Lactobacillus fructivorans*. Maltose is present to allow the growth of lactobacilli which cannot utilise glucose. The media can be made selective against yeasts by the addition of 7mg/l cycloheximide (Actidione®) and against Gram-negative bacteria by the addition of 3g/l 2-phenylethanol.

| Typical Formula | g/litre |
|---|---|
| Yeast extract | 5.0 |
| Tryptone | 20.0 |
| Liver concentrate | 1.0 |
| Maltose | 10.0 |
| Fructose | 5.0 |
| Dextrose | 5.0 |
| Betaine HCl | 2.0 |
| Diammonium hydrogen citrate | 2.0 |
| Potassium aspartate | 2.5 |
| Potassium glutamate | 2.5 |
| Magnesium sulphate $7H_2O$ | 2.0 |
| Manganese sulphate $4H_2O$ | 0.66 |
| Potassium phosphate | 2.0 |
| N-acetyl glucosamine | 0.5 |
| Agar | 17.0 |

**Method for reconstitution**

Disperse 77.1g of powder in 1 litre of distilled water. Add 10ml Sorbitan mono-oleate and 7mg cycloheximide. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121°C. Cool to 50°C. If required, aseptically add 3g of 2-phenylethanol. Mix well and pour into sterile Petri dishes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear to slightly opalescent amber coloured gel

**pH:** 5.4 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Lactobacillus fermentum* ATCC 9338
*Pediococcus acidilactici* NCTC 6990
*Escherichia coli* ATCC 25922 (inhibited / suppressed)

**Inoculation:**

Surface technique

Spread 0.1ml of the sample over the surface of the agar. Alternatively, the sample may be filtered and the membrane placed on the surface of the agar.

Overlay technique

Aseptically dispense 4ml volumes of Raka-Ray No.3 Agar into test tubes and keep molten at 50°C. Mix 1ml of the test sample with 4ml of molten agar and immediately pour the contents into a Petri dish containing 15-20ml Raka-Ray No.3 Agar. Mix to give isolated colonies. As the agar layer is very thin, individual colonies can be picked for further examination.

**Incubation:** Incubate anaerobically at 25-30°C for 7 days.

**Interpretation:** Lactobacilli are visible after 48 hours incubation and appear as smooth, cream-coloured, moist colonies approximately 1mm in diameter.

Incubation for 4 days may be sufficient, however slow-growing organisms such as *Pediococcus* may require upto 7 days.

If the number of colonies on the plate exceeds 300, dilute the sample 1:10 in Maximum Recovery Diluent (LAB103) and retest.

**References**


Coster, E., and White, H.R. (1951). J. Gen. Microbiol. 37:15.

European Brewing Convention, EBC *Analytica Microbiologica: Part II J. institute of Brewing* (1981) 87. 303-321.

Lawrence D. R. and Leedham P. A. (1979) *Journal of the Institute of brewing* 85. 119

Mauld B. and Seidel H. (1971) *Brauwissenschaft* 24, 105

Methods of Analysis of the American Society of Brewing Chemists ASBC (1976) 7th edition, The Society St. Paul. Mn. USA.

Saha R. B., Sondag R. J. AND Middlekauff J. E. (1974). *Proceedings of the American Society of Brewing Chemists*, 9th Congress 1974.

Van Keer C., Van Melkebeke l., Vertrieste W., Hoozee g. and Van Schoonenberghe E. (1983). *Journal of the Institute of brewing* 89. 361 – 363.


# *Raka-Ray No.3 Agar (Increased Gel Strength)*

**LAB199**

**Description**

Based on the formulation of Saha, Sondag and Middlekauff, Raka-Ray No.3 Agar is for the detection of lactic acid bacteria in beer and for monitoring in-process beer quality. It is recommended for this application by the European Brewing Convention (EBC) and the American Society of Brewing Chemists (ASBC). LAB199 Raka-Ray No.3 Agar Increased Gel Strength contains the same components as LAB198 Raka-Ray No.3 Agar, however the agar level has been increased to 27g/l.

Contamination of beer and the beer making process by members of the lactobacilli family results in spoilage, primarily through their production of metabolic products which are detrimental to the flavour of the final product. Detection of these organisms is complicated by their diverse nutritional and environmental requirements.

A number of different formulations have been described for the isolation of lactic acid bacteria in brewing products and processes. Raka-Ray Agar was developed by the addition of various growth promoting compounds to Universal Beer Agar. This work led to the recognition that the addition of sorbitan mono-oleate, liver extract and N-acetylglucosamine produced superior growth when compared to the standard Universal Beer Agar formulation.

Further investigations provided the basis for the final formula of Raka-Ray No. 3 Medium in which fructose is an essential carbohydrate source for *Lactobacillus fructivorans*. Maltose is present to allow the growth of lactobacilli which cannot utilise glucose. The media can be made selective against yeasts by the addition of 7mg/l cycloheximide (Actidione®) and against Gram-negative bacteria by the addition of 3g/l 2-phenylethanol.

Due to customer feedback regarding the soft gel strength of the standard formulation, Lab M has developed this modified version of Raka-Ray Agar with a higher gel strength. The increased gel strength is especially useful when performing surface plating techniques.

| Typical Formula | g/litre |
|---|---|
| Yeast extract | 5.0 |
| Tryptone | 20.0 |
| Liver concentrate | 1.0 |
| Maltose | 10.0 |
| Fructose | 5.0 |
| Dextrose | 5.0 |
| Betaine HCl | 2.0 |
| Diammonium hydrogen citrate | 2.0 |
| Potassium aspartate | 2.5 |
| Potassium glutamate | 2.5 |
| Magnesium sulphate $7H_2O$ | 2.0 |
| Manganese sulphate $4H_2O$ | 0.66 |
| Potassium phosphate | 2.0 |
| N-acetyl glucosamine | 0.5 |
| Agar | 27.0 |

**Method for reconstitution**

Disperse 77.1g of powder in 1 litre of distilled water. Add 10ml Sorbitan mono-oleate and 7mg cycloheximide. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121°C. Cool to 50°C. If required, aseptically add 3g of 2-phenylethanol. Mix well and pour into sterile Petri dishes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear to slightly opalescent amber coloured gel

**pH:** 5.4 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Lactobacillus fermentum* ATCC 9338
*Pediococcus acidilactici* NCTC 6990
*Escherichia coli* ATCC 25922 (inhibited / suppressed)

**Inoculation:**

Surface technique

Spread 0.1ml of the sample over the surface of the agar. Alternatively, the sample may be filtered and the membrane placed on the surface of the agar.

Overlay technique

Aseptically dispense 4ml volumes of Raka-Ray No.3 Agar into test tubes and keep molten at 50°C. Mix 1ml of the test sample with 4ml of molten agar and immediately pour the contents into a Petri dish containing 15-20ml Raka-Ray No.3 Agar. Mix to give isolated colonies. As the agar layer is very thin, individual colonies can be picked for further examination.

**Incubation:** Incubate anaerobically at 25-30°C for 7 days.

**Interpretation:** Lactobacilli are visible after 48 hours incubation and appear as smooth, cream-coloured, moist colonies approximately 1mm in diameter.

Incubation for 4 days may be sufficient, however slow-growing organisms such as *Pediococcus* may require upto 7 days.

If the number of colonies on the plate exceeds 300, dilute the sample 1:10 in Maximum Recovery Diluent (LAB103) and retest.

**References**


Coster, E., and White, H.R. (1951). J. Gen. Microbiol. 37:15.

European Brewing Convention, EBC *Analytica Microbiologica: Part II J. institute of Brewing* (1981) 87. 303-321.

Lawrence D. R. and Leedham P. A. (1979) *Journal of the Institute of brewing* 85. 119

Mauld B. and Seidel H. (1971) *Brauwissenschaft* 24, 105

Methods of Analysis of the American Society of Brewing Chemists ASBC (1976) 7th edition, The Society St. Paul. Mn. USA.

Saha R. B., Sondag R. J. AND Middlekauff J. E. (1974). *Proceedings of the American Society of Brewing Chemists*, 9th Congress 1974.

Van Keer C., Van Melkebeke l., Vertrieste W., Hoozee g. and Van Schoonenberghe E. (1983). *Journal of the Institute of brewing* 89. 361 – 363.


## *Rappaport Vassiliadis (R.V.) Medium*

**LAB086**

**Introduction**

Rappaport Vassiliadis Broth (R10 modification) was born out of a long series of experiments carried out to determine the correct levels of malachite green and magnesium chloride that would allow *Salmonella* to multiply freely yet still inhibit the other enteric organisms.

This formulation has been shown to be superior to Mueller Kauffmann and Selenite Broth for the isolation of *Salmonella* from meat products.

The development work carried out on the formulation shows that it is extremely efficient in detecting small numbers of *Salmonella* in heavily contaminated products. This formulation is very hygroscopic and will produce a slight exothermic reaction when mixed with water.

| Typical Formula | g/litre |
|---|---|
| Soy Peptone | 4.5 |
| Sodium chloride | 7.2 |
| Potassium dihydrogen phosphate | 1.26 |
| Dipotassium hydrogen phosphate | 0.18 |
| Magnesium chloride anhydrous | 13.58 |
| Malachite green | 0.033 |

**Method for reconstitution**

Weigh 26.8 grams powder, disperse in 1 litre of deionised water, swirl to mix, when dissolved dispense in 10ml volumes in screw capped bottles and sterilise by autoclaving at 115˚C for 15 minutes.

**Appearance:** Clear, blue fluid.

**pH:** 5.2 ± 0.2

Minimum Q.C. organisms: *E. coli* (inhibited) WDCM 00013
*S. typhimurium* WDCM 00031

**Storage of Prepared Medium:** Capped container – 6 months at 2-8˚C

**Inoculation:** From pre-enrichment broth in the proportions of 1-part inoculum to 99 parts R.V. Broth. Sub-culture onto either XLD Agar, M.L.C.B. Agar or other salmonella selective agars.

**Incubation:** 41.5 ± 0.5˚C for 24 hours (incubator) or 42 ± 0.1˚C for 24hrs (water bath).

**References**


Vassiliadis, P., (1983) The Rappaport Vassiliadis (R.V.) Enrichment Medium for the Isolation of salmonellas: An overview J. Appl. Bacteriol. 56 69-76.

Vassiliadis, P., Mavromatti, CH. Efstratiou, M. and Chronas, G. (1985). A note on the stability of Rappaport-Vassiliadis Enrichment Medium J. Appl. Bacteriol. 59 143-145.

Bolton, F.G., Preston, P.H.L. Personal communication.

Int. J. Food Micro. Pharmacopoeia of culture media for Food Microbiology.

Peterz, M., Wiberg, C., and Norberg, P. 1989. The effect of incubation temperature and magnesium chloride concentration on growth of *Salmonella* in home-made and in commercially available dehydrated Rappaport-Vassiliadis broths.


## *Reinforced Clostridial Agar*

**LAB023**

**Description**

This is a solidified version of R.C.M. (LAB022) and can be used for the enumeration of anaerobes by pour plate, shake tube or membrane filtration methods. When solidified in tubes or bottles with minimal head space it can be used for anaerobic culture without the need for anaerobic atmosphere.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 3.0 |
| Beef Extract | 10.0 |
| Peptone | 10.0 |
| Glucose | 5.0 |
| Soluble Starch | 1.0 |
| Sodium chloride | 5.0 |
| Sodium acetate | 3.0 |
| L-Cysteine hydrochloride | 0.5 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 49.5 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 47˚C. and distribute into sterile dishes or tubes containing decimal dilutions of the sample under test.

**Appearance:** Pale straw, translucent gel.

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms: *C. perfringens* WDCM 00007

**Storage of Prepared Medium:** Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Pour plate technique or tube culture.

**Incubation:** 30˚C for up to 72 hours. Anaerobic conditions for pour plate. Count as early as possible as prolonged incubation may result in the medium being disrupted due to gas production.

**Interpretation:** Count all colonies as presumptive clostridia.

**References**

Miller, N.J., Garrett, O.W. and Prickett, P.S. (1939). Anaerobic technique – a modified deep agar shake. Food Res. 4: 447-451.

Ingram, M. and Barnes, E.M. (1956). A simple modification of the deep shake tube for counting anaerobic bacteria. Lab. Pract. 5: 145.

## *Reinforced Medium for Clostridia (USP/EP/JP)*

**HP011**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the selective enrichment of *Clostridia* from non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. The medium is also commonly referred to as Reinforced Clostridial Medium and abbreviated to RCM. Peptone, beef and yeast extract provide a source of nitrogen, essential vitamins and amino acids. Starch aids the detoxification of harmful metabolites and glucose is a fermentable carbohydrate. Sodium chloride provides osmotic balance and sodium acetate acts as a buffer. L-Cysteine act as reducing agents to create an anaerobic environment and maintain a low Eh. This is aided by the low level of agar which reduces the oxygen permeability through the medium. According to the Harmonised European Pharmacopoeia, Reinforced Medium for Clostridia is used as a selective enrichment broth, with subculture performed onto Columbia Agar.

| Typical Formula | g/litre |
|---|---|
| Beef extract | 10.0 |
| Peptone | 10.0 |
| Yeast extract | 3.0 |
| Soluble starch | 1.0 |
| Glucose monohydrate | 5.0 |
| Cysteine hydrochloride | 0.5 |
| Sodium chloride | 5.0 |
| Sodium acetate | 3.0 |
| Agar | 0.5 |

**Method for reconstitution**

Disperse 38 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and bring to the boil. Distribute into suitable vessels and sterilise at 121°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw, clear to slight haze

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms:
*Clostridium sporogenes* ATCC 19404

**Hazard classification:** NR - Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the Harmonised European Pharmacopoeia, a sample is prepared with and without heat treatment and transferred to Reinforced Medium for Clostridia.

**Incubation:**

Incubate at 30-35°C for 48-72 hours

**Growth Characteristics**

| Organism | Expected result in RCM | Expected result on Columbia Agar subculture |
|---|---|---|
| *Clostridia spp.* | Growth | Growth, typical colonies |

**References**

European Pharmacopoeia 8th Edition

## *Rhamnose MacConkey (VTEC O26) Agar (RMAC)*

**LAB209**

**Description**

This medium is selective for the isolation of the verocytotoxin producing *Escherichia coli* O26. This strain has been associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). It is based on the MacConkey formulation No.3, where the fermentable carbohydrate of lactose has been substituted for rhamnose. VTEC O26 colonies are not able to ferment the rhamnose so will remain a translucent colour on the medium. All other non-O26 VTEC colonies present are able to ferment Rhamnose and will appear as pink to red colonies. Selectivity of the medium can be increased by adding X161 Cefixime Tellurite (CT) supplement.

| Typical Formula | g/litre |
|---|---|
| Peptone | 15.0 |
| Rhamnose | 20.0 |
| Bile salts No.3 | 1.5 |
| Sodium chloride | 5.0 |
| Neutral red | 0.03 |
| Crystal violet | 0.001 |
| Agar No.2 | 12.0 |

**Method for reconstitution**

Weigh 53.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C, and add 2 vials of reconstituted X161. Mix well and dispense into sterile Petri dishes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: red/purple gel

**pH:** 7.1 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Escherichia coli* NCTC 8783
*Escherichia coli* WDCM 00013
*Salmonella* typhimurium WDCM 00031
*Staphylococcus aureus* WDCM 00034
*Enterococcus faecalis* WDCM 00087

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight

Poured plates: 7 days at 2-8°C in the dark

**Inoculation:** Surface inoculation as per user's validated method.

**Incubation:** Incubate at 37°C for 18-24 hours.

**References**

Hiramatsu R, Matsumoto M, Miwa Y, Saito M, Yatsuyanagi J, Uchimura M, Kobayashi K, Tanaka H, Horikawa K, Mori R, Miyazaki Y. Characterization of enterohemorrhagic Escherichia coli O26 and development of its isolation media. Kansenshogaku Zasshi. 1999 May; 73(5):407-13.

Hiramatsu, R., Matsumoto, M., Miwa, Y., Suzuki, Y., Saito, M., and Miyazaki, Y. Characterization of Shiga Toxin-Producing Escherichia coli O26 Strains and Establishment of Selective Isolation Media for These Strains. J Clin Microbiol. 2002 March; 40(3): 922–925.

## *Ringer's Solution (1/4 strength) Tablets*

**LAB100Z**

**Description**

An osmotically controlled solution for the preparation of suspensions of food samples and for use as a diluent in dilution techniques for bacterial enumeration. The solution can also be used in the sampling of food production apparatus by the rinse and swab method.

| Typical Formula | g/litre |
|---|---|
| Sodium chloride | 2.25 |
| Potassium chloride | 0.105 |
| Calcium chloride | 0.12 |
| Sodium bicarbonate | 0.05 |

**Method for reconstitution**

Dissolve 1 tablet in 500ml deionised water. When completely dissolved dispense into containers as required and sterilise by autoclaving for 15 minutes at 121°C.

Minimum QC organisms: *Escherichia coli* WDCM 00013
*Staphylococcus aureus* WDCM 00031

**Appearance:** Tablet: white tablet

Finished medium: colourless, clear liquid

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

## *Rose Bengal Chloramphenicol Agar Base*

**LAB036**

**Description**

A selective medium for the enumeration of moulds and yeasts in foods. The original formulation of Jarvis (1973) used chlortetracycline, this has been substituted by chloramphenicol because of superior selectivity. The Rose Bengal dye is taken up by the growing colonies making them easier to see and inhibiting their spreading. Rose Bengal becomes increasingly toxic on exposure to light so it is important to store plates in the dark.

| Typical Formula | g/litre |
|---|---|
| Mycological Peptone | 5.0 |
| Dextrose | 10.0 |
| Dipotassium phosphate | 1.0 |
| Magnesium sulphate | 0.5 |
| Rose bengal | 0.05 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 28.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121°C. Allow to cool to 47°C then add 2 vials of X009 (or 1 vial of X209) Chloramphenicol. X089 Oxytetracycline (2 vials per litre) may also be used. Mix well, then pour into Petri dishes.

This medium should be protected from light. Chloramphenicol may be added before autoclaving.

DO NOT REHEAT THIS MEDIA ONCE PREPARED.

**Appearance:**

Powder: fine, free-flowing, homogeneous, pink
Finished medium: Pink gel

**pH:** 7.2 ± 0.2

**Hazard classification:** NR – Not regulated

Minimum Q.C. organisms:
*Aspergillus niger* WDCM 00053
*Saccharomyces cerevisiae* WDCM 00058
*Escherichia coli* WDCM 00013 (inhibition)

**Storage of Prepared Medium:**
Dehydrated culture media: 10-25°C
Prepared media: Plates - up to 7 days at 2-8°C, in the dark
Capped containers – up to 1 month at 2-8°C, in the dark

**Inoculation:** Surface spreading.

**Incubation:** 25°C aerobically for 24 hours to 5 days.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Rhizopus* spp. | 13.5 | Fluffy | White | |
| *Aspergillus flavus* | 8 | Flat & hyphae | Yellow/ Green | hyphae |
| *Candida* spp. | 1.5 | CV.E.G. | White | |
| *Sacchromyces* spp. | 1.0 | CV.E.G. | White | |
| *E. coli* | no growth | | | |
| *Staphylococcus* spp. | no growth | | | |

**References**


Banks, J.G. Board, R.G. (1987). Some factors influencing the recovery of yeasts and moulds from chilled foods. Int. J. Food Microbiol. 4: 197-206.

Jarvis, B. (1973). Comparison of an improvised Rose-Bengal Chlortetracycline Agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J. Appl. Bact. 36: 723-727.

Overcast, W.W. and Weakley, D.L. (1969). An aureomycin rose-bengal agar for the enumeration of yeast and mould in cottage cheese. J.Milk and Fd.Tech. 32: 442-445.


## *Sabouraud Dextrose Agar*

**LAB009**

**Description**

Introduced by Sabouraud in 1910 as a selective medium for fungi and yeasts. The acidic pH (5.6) of this medium inhibits many species of bacteria. The medium can be made more selective by the addition of chloramphenicol supplement (X009) (X209). Diagnostic features, such as sporing structures and pigmentation are well developed on this medium. Because of its low pH this medium is very sensitive to overheating which will soften the agar and caramelise the carbohydrate.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 10.0 |
| Dextrose | 40.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 62 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving for 15 minutes at 121°C. NOTE: The gel strength of the medium may diminish if recommended sterilising time or temperature is exceeded.

Cool to 47°C, mix well then pour plates.

**Appearance:** Buff opalescent gel.

**pH:** 5.6 ± 0.2

Minimum Q.C. organisms: *Candida sp.* WDCM 00054
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation method:** Surface streaking for single colonies or stab method.

**Incubation:** Aerobically, yeasts 37˚C for 48 hours; fungi 25-30˚C for up to 3 weeks.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *C. albicans* | 0.5-2.0 | CV.E.D. | White | Yeasty smell |
| *C. krusei* | 1.0-3.0 | F.CR.D. | Grey-White | Yeasty smell |
| *T. rubrum* | 25 | White-fluffy | Reverse-shades of red | |
| *M. canis* | 25 | White-centre-yellow radial | reverse-yellow- | |

**References**

Sabouraud, R. (1910). Les Teignes Paris. Pagano. J., Levin, J.D. and Trejo, W. (1957-8). Diagnostic medium for the differentiation of species of Candida. Antibiotics Annual, 137-143.

## *Sabouraud Dextrose Broth (USP/EP/JP)*

**HP013**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the enrichment of *Candida albicans* from non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. The medium is also used for the cultivation of fungal test strains as described by the Harmonised European Pharmacopoeia. The peptone digests and dextrose provide a nutritious base for luxuriant fungal growth and the acidic pH affords selectivity against bacteria. Due to the high carbohydrate content and low pH this medium is highly sensitive to overheating. According to the Harmonised European Pharmacopoeia, Sabouraud Dextrose Broth is used as an enrichment broth, with subculture performed onto Sabouraud Dextrose Agar.

| Typical Formula | g/litre |
|---|---|
| Dextrose | 20.0 |
| Peptic digest of animal tissue | 5.0 |
| Pancreatic digest of casein | 5.0 |

**Method for reconstitution**

Disperse 30 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Distribute into suitable vessels and sterilise at 121°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw, clear to slight haze

**pH:** 5.6 ± 0.2

Minimum Q.C. organisms:
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR - Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the Harmonised European Pharmacopoeia, a quantity corresponding to 1g or 1mL of the sample is use to inoculate 100mL of Sabouraud Dextrose Broth.

**Incubation:**

Incubate at 30-35°C for 3-5 days

**Growth Characteristics**

| Organism | Expected result in SDB | Expected result on SDA subculture |
|---|---|---|
| *Candida albicans* | Growth | Growth, white colonies |

**References**

European Pharmacopoeia 8th Edition

## *Sabouraud Maltose Agar*

**LAB111**

**Description**

This is a modification of Sabouraud Dextrose Agar, substituting maltose for dextrose, recommended by the American Public Health Association.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone | 10.0 |
| Maltose | 40.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 62 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then autoclave at 121˚C for 15 minutes. Do not overheat or the agar gel will be softened and the carbohydrate will be caramelised. This medium may be made selective by the addition of 2 ampoules X009 Chloramphenicol selective supplement which may be added either before or after autoclaving. Cool to 47˚C and mix well before pouring plates.

**Appearance:** Cream/yellow, translucent.

**pH:** 5.6 ± 0.2

Minimum Q.C. organisms: *Candida albicans* WDCM 00054
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Surface streaking or stab inoculum.

**Incubation:** Aerobic; yeasts 37˚C for 48 hours; other fungi 25˚C for up to 3 weeks.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *C. albicans* | 0.5-2.0 | CV.E.D. | White | Yeasty smell |
| *C. krusii* | 1.0-3.0 | F.CR.D. | Grey-White | Yeasty smell |
| *T. rubrum* | 25mm | White-fluffy | Reverse-shades of Red | |
| *M. canis* | 25mm | White-centre yellow radial | Reverse-yellow-orange | |

**References**

Sabouraud, R. (1910). Les Teignes. Paris. Pagano, J., Levin, J. D. and Trejo, W. (1957-8) Diagnostic medium for the differentiation of species of Candida. Antibiotics Annual. 137-143.

# *Selenite Broth*

**LAB044A & LAB044B**

**Description**

A medium for the selective enrichment of salmonellae from faeces, food and sewage. First described by Leifson in 1936 the medium is a peptone lactose broth, moderately buffered, which utilises sodium biselenite as a selective agent. This medium can be incubated at various temperatures from 35-43˚C to vary the selectivity. Subcultures should be performed after no more than 24 hours incubation as there is an increasing loss of selectivity if incubation is prolonged.

| Typical Formula | g/litre |
|---|---|
| **Selenite Broth Base LAB044A:** | |
| Peptone | 5.0 |
| Lactose | 4.0 |
| Sodium phosphate | 10.0 |
| **Sodium biselenite LAB044B:** | |
| Sodium hydrogen selenite | 4.0 |

**Method for reconstitution**

Dissolve 4 grams of sodium biselenite in 1 litre of cold deionised waer. Add 19 grams of Selenite Broth Base and warm to dissolve. Distribute into tubes or bottles and sterilise for 5-10 minutes in a boiling water bath, or by free steaming. DO NOT AUTOCLAVE.

**Appearance:** Pale orange/red with slight precipitate (overheating will cause excessive precipitate and loss of selectivity).

**pH:** 7.1 ± 0.2 (complete medium)

Minimum Q.C. organisms:
*Salmonella typhimurium* WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Approximately 0.5-1 gram of sample per 10ml tube.

**Incubation:** Up to 24 hours aerobically at 35-43˚C.

**Subculture:** Onto two or more selective agars.

**References**


Leifson, E. (1936). New selenite enrichment media for isolation of typhoid and paratyphoid *(Salmonella)* bacilli. Amer. J.Hyg. 24: 423-432.

MacFaddin, J.F. (1985). Media for the isolation, cultivation, identification of Medical Bacteria Vol 1. Williams and Wilkins, Baltimore.


# *Selenite Cystine Broth*

**LAB055A & LAB044B**

**Description**

This formulation is a result of the investigation of North and Bartram in 1953. They examined the effect of varying concentrations of cystine and phosphate on the recovery of salmonellae in egg products using selenite broth. It was found that the addition of 10 micrograms/ml of cystine to Leifson's selenite broth enhanced recovery of salmonellae.

| Typical Formula | g/litre |
|---|---|
| **Selenite Cystine Broth Base LAB055A** | |
| Balanced Peptone No. 1 | 5.0 |
| Lactose | 4.0 |
| Sodium phosphate | 10.0 |
| L-Cystine | 0.01 |
| **Sodium biselenite LAB044B** | |
| Sodium hydrogen selenite | 4.0 |

**Method for reconstitution**

Dissolve 4 grams of Sodium biselenite (LAB044B) in 1 litre of deionised water. Add 19 grams of Selenite Cystine Broth Base and heat to dissolve. Distribute into tubes or bottles, and sterilise for 10 minutes in a boiling water bath, or steamer. DO NOT AUTOCLAVE THIS MEDIUM.

**Appearance:** Pale straw colour, clear with slight precipitate. (A brick red precipitate indicates overheating).

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms:
*Salmonella typhimurium* WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Add sample to broth in the ratio of 1:10. Use a pre-enrichment broth if damaged organisms are to be recovered.

**Incubation:** 37˚C for 24-48 hours aerobically. Subculture onto *Salmonella* selective media.

**References**


International Organisation for Standardization. Microbiology (1981). General guidance on methods for the detection of *Salmonella.* ISO, 6579-1981.

International Organization for Standardization. Milk and milk products (1985). Detection of *Salmonella.* ISO 6785-2985 (E).

ICMSF, (1978). Micro-organisms in foods. 1. Their significance and methods of enumeration, 2nd edn., University of Toronto Press, Toronto, Ont.

Leifson, E. (1936). New selenite enrichment media for the isolation of typhoid and paratyphoid *(Salmonella)* bacilli. Am, J. Hyg. 24, 423-432.

North, W.R. and. Bartram, M.T. (1953). The efficiency of selenite broth of different compositions in the isolation of *Salmonella.* Appl. Microbiol. 1, 130, 134.

Speck, M.L. (1984). Compendium of methods for the microbiological examination of foods, 2nd edn., American Public Health Association.


# *Sensitivity Test Agar*

***(S.T.A.)***

**LAB012**

**Description**

A medium formulated for antibiotic susceptibility testing by the Joan Stokes technique. S.T.A. is inhibitor-free, very rich and includes various nucleotides to enable fastidious organisms to be tested. It is necessary to add lysed or 'chocolated' blood for some organisms.

| Typical Formula | g/litre |
|---|---|
| Peptone-Infusion Solids | 21.5 |
| Starch | 0.6 |
| Sodium chloride | 5.0 |
| Disodium citrate | 1.0 |
| Adenine sulphate | 0.01 |
| Guanine hydrochloride | 0.01 |
| Uracil | 0.01 |
| Xanthine | 0.01 |
| Aneurine hydrochloride | 0.01 |
| Uridine | 0.1 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 40 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise by auto-claving for 15 minutes at 121˚C. To prepare blood agar cool to 45˚C and add 7% lysed blood or 6% defibrinated blood according to preference. Mix well then pour plates.

**Appearance:** Dependent upon the blood additive.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* NCTC 6571
*E. coli* NCTC 10418
(antibiotic sensitivity zones)

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation method:** Surface, according to technique.

**Incubation:** 37˚C, atmosphere to suit organisms metabollic requirements.

**Interpretation:** There are no defined zone sizes as in Mueller Hinton, but all antibiotics should give adequate zone sizes when compared to controls using standard organisms, e.g. *S. aureus* NCTC 6571, *E. coli* NCTC 10418, *Ps. aeruginosa* NCTC 10662.

**References**

Stokes, E.J. (1968). Clinical Bacteriology 3rd edn. Arnold, London. Committee of the A.C.P. (1965). Report on the Antibiotic Sensitivity test trial organised by the bacteriology committee of the Association of Clinical Pathologists. J.Clin. Pathol., 18: 1-5.

Hanus, F.J. Sands, J.G. and Bennett, E.O. (1967). Antibiotic activity in the presence of agar. Appl. Microbiol., 15: 31-34.

Bechtle, R.M. and Scherr, G.H. (1958). A new agar for in vitro antimicrobial sensitivity testing. Antibiot. Chemother., 8: 599-606.

## *Simmons Citrate Agar*

**LAB069**

**Description**

A medium devised by Simmons in 1926 to help in the differentiation of enteric bacteria and in the isolation of fungi. Certain *Enterobacteriacae* have the ability to utilize citrate as the sole source of carbon and utilize inorganic ammonium salts as the sole source of nitrogen resulting in an increase in alkalinity. Bromothymol Blue is used as a pH indicator.

| Typical Formula | g/litre |
|---|---|
| Magnesium sulphate | 0.2 |
| Ammonium dihydrogen phosphate | 1.0 |
| Dipotassium phosphate | 1.0 |
| Sodium citrate | 2.0 |
| Sodium chloride | 5.0 |
| Bromothymol blue | 0.08 |
| Agar No. 2 | 15.0 |

**Method for reconstitution**

Weigh 24 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then heat to dissolve the agar and solids. Dispense into tubes or bottles then sterilise by autoclaving at 121˚C for 15 minutes. Allow to set as slopes.

**Appearance:** Green, opalescent.

**pH:** 6.9 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*C. freundii* WDCM 00006

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Streak on surface and stab into the butt.

**Incubation:** 37˚C aerobically for 24-48 hours, with loose caps to allow gaseous exchange.

**Interpretation:** Utilisation of citrate and ammonium salts results in growth and a change in colour of the medium from green to blue.

**Growth Characteristics**

| organism | growth | colour of medium |
|---|---|---|
| (most) *Salmonella* spp | yes | blue |
| *Shigella* spp. | no | green |
| *E. coli* | no | green |
| *C. freundii* | yes | blue |

**References**

Simmons, J.S. (1926). A Culture medium for differentiating organisms of typhoid - colon aerogenes groups and for isolation of certain fungi. J.Inf. Dis. 39: 209-215.

Koser, S.A. (1923). Utilisation of the salts of organic Acids by the Colon-aerogenes group. J. Bact. 8: 493-520.

MacFaddin, J.F. (1983). Biochemical Tests for Identification of Medical Bacteria. Williams and Wilkins.

## *Slanetz and Bartley Medium*

***(Membrane Enterococcus Agar)***

**LAB166**

**Description**

This medium was originally described by Slanetz and Bartley for the enumeration of enterococci from water samples using a membrane filtration technique, but it may also be used as a spread plate for the examination of other sample types. Enterococci reduce tetrazolium chloride to the insoluble red dye formazan, producing colonies which are dark red or maroon on the surface of the membrane or agar. This reaction is not exclusive to enterococci, and the count at this stage should be considered presumptive. Colonies may be confirmed as enterococci by demonstrating aesculin hydrolysis using Kanamycin Aesculin Azide Agar LAB106.

| Typical Formula | g/litre |
|---|---|
| Tryptose | 20.0 |
| Yeast Extract | 5.0 |
| Glucose | 2.0 |
| Dipotassium hydrogen phosphate | 4.0 |
| Sodium azide | 0.4 |
| 2,3,5 Tetrazolium chloride | 0.1 |
| Agar | 12.0 |

**Method for reconstitution**

Weigh 43.5 grams of powder and mix with 1 litre of deionised water. Bring to the boil with frequent stirring to dissolve completely. Cool to 47˚C and pour into sterile Petri dishes. DO NOT AUTOCLAVE, OVERHEAT, OR LEAVE FOR GREATER THAN 4hr AT 47˚C.

**Appearance:** Rose coloured gel.

**pH:** 7.2 ± 0.2

Mininmum QC organisms:
*Enterococcus faecalis* WDCM 00087
*Escherichia coli* WDCM 00013 (inhibition)

**Storage:** Plates – upto 7 days at 2-8˚C. Storage in bottles is not recommended as re-melting the medium will cause damage.

**Inoculation**

Water: Filter 100ml of the water through a suitable membrane, and place this on the surface of a properly dried Slanetz and Bartley plate.

Other samples: Dilute as necessary and spread 0.5ml over the surface of the plate using a spreader, and allow to soak into the agar.

**Incubation**

Water: at 37˚C for 48hr if testing potable waters or processed foods. At 37˚C for 4hr then 44˚C for 44hr if testing untreated waters or raw materials.

**Interpretation**

Count all red and maroon colonies as presumptive enterococci. Confirmation of isolates can be achieved by demonstration of a positive aesculin reaction on KAAA LAB106.

**Reference:**

Slanetz, L.W., and Bartley, C.H. (1957) J.Bact. **74** 591-595.

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

# *Sorbitol MacConkey Agar*

***(SMAC, CT-SMAC)***

**LAB161**

**Description**

This is a selective differential medium for the isolation of *Escherichia coli* O157:H7, the primary serovar associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Pathogenicity of the organism is linked to the production of verocytotoxins (VT1 and VT2), but it should be noted that not all strains of O157:H7 produce verocytotoxins, and that strains from other serovars can be toxin producers (e.g. O26, O111, O113, O145).

O157:H7 has been associated epidemiologically with food poisoning outbreaks involving beefburgers and cold cooked meats. The medium is a modification of MacConkey Agar No. 3 with the substitution of the fermentable carbohydrate from lactose to sorbitol. O157:H7 is sorbitol negative and produces translucent colonies whereas most other *E. coli* strains are sorbitol positive and so produce pink/red colonies. Selectivity of the medium can be increased by adding Cefixime-Tellurite (C.T.) supplement X161.

| Typical Formula | g/litre |
|---|---|
| Peptone | 20.0 |
| Sorbitol | 10.0 |
| Bile salts no.3 | 1.5 |
| Sodium chloride | 5.0 |
| Neutral red | 0.03 |
| Crystal violet | 0.001 |
| Agar | 12.0 |

**Method for reconstitution**

Weigh 48.5 grams of powder and add to 1 litre of de-ionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C, add 2 vials of CT supplement X161, and pour plates. Dry the surface prior to inoculation.

**Appearance:** Pale red, slight violet tinge.

**pH:** 7.1 ± 0.2

Minimum QC organisms:
*E. coli* O157:H7 (non-toxigenic) WDCM 000014 (translucent)
*E. coli* WDCM 00013 (Pink/red)
*Ent. faecalis* WDCM 00087 (inhibition)

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface streak for single colonies.

**Incubation:** 37˚C aerobically for 18-24 hr.

**Growth Characteristics:**

| Organism | Size (mm) | Shape | Colour |
|---|---|---|---|
| *E. coli* O157:H7 | 2.5 – 4.0 | CV.E.G | Translucent |
| Other *E. coli* | 2.5 – 4.0 | CV.E.G | Pink/red |
| Sorbitol +ve organisms | 2.5 – 5.0 | Any | Pink/red |

**References**


Law, D., Ganguli, L.A., Donohue-Rolfe, A., Acheson, D.W.K. (1992) J. Med. Micro. 36 198-202.

Hitchins, A.D., Hartman, P.A., and Todd, E.C.D. (1992) in "Compendium of methods for the microbiological examination of foods" Ch.24. Published by American Public Health Association.

Varnam, A.H., Evans, M.G., (1991) Foodborne Pathogens an Illustrated Text. Published by Wolfe Publishing Ltd.

Riley, L.W. (1991) Ann. Rev. Micro. 41 383-408.

Riley, L.W. *et al* (1983) New Eng. J. Med 308 681-685.

Salmon, R.L., Farrel, I.D., Hutchinson, J.G.P. (1989) Epid. Inf. 103 249-254.


# *S.S. Agar*
# *(Salmonella Shigella Agar)*

**LAB052**

**Description**

This medium is a modification of Leifson's DCA Medium first described in 1941 by Mayfield and Goeber shortly before Hynes described his modification of DCA. The selectivity of the medium was increased by the addition of extra bile salts, sodium citrate and the addition of brilliant green dye. There is also the extra thiosulphate giving good $H_2S$ production which reduces the ferrous ammonium sulphide giving black centred colonies with $H_2S$ positive organisms.

The selectivity of this medium can be such that it was suggested by Taylor *et al* in 1965 to be unsuitable for the isolation of *Shigella* species. Greater understanding of the selection mechanisms involved enable us to adjust the reaction and allow the more delicate *Shigella* to grow without unduly impairing the medium's selective properties.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 5.0 |
| Balanced Peptone No. 1 | 5.0 |
| Lactose | 10.0 |
| Bile Salts No. 3 | 8.5 |
| Sodium citrate | 8.5 |
| Sodium thiosulphate | 8.5 |
| Ferric citrate | 1.0 |
| Brilliant Green | 0.00033 |
| Neutral Red | 0.025 |
| Agar No. 2 | 13.5 |

**Method for reconstitution**

Weigh 60 grams of powder, disperse in 1 litre of deionised water, and allow to soak for 10 minutes. Swirl to mix, then bring to the boil, and allow to cool to 47˚C. Mix well then pour plates. Dry the surface before incubation. DO NOT AUTOCLAVE THIS MEDIUM.

**Appearance:** Pale Pink, clear.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms:
*Salmonella typhimurium* WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 4˚C in the dark.

**Inoculation method:** Surface plating, streaking for single colonies.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *E. coli* | 0.1-2.0 | CV.E.D. | Red | Red ppt around colonies (No growth) |
| *K. aerogenes* | 0.1-3.0 | CV.E.G. | Pink-Red | (No growth) |
| *Proteus* spp. | 1.0-2.0 | CV.E.G. | Yellow | (grey centre) (fishy odour) |
| *Salmonella* spp. | 2.0-3.0 | CV.E.G. | Yellow | (black centre) |
| *Shigella* spp. | 0.5-2.0 | CV.E.G. | Pink-Yellow | |

Gram positive organisms – no growth.

**References**


Isenberg, H.D., Kominos, S., and Siegel, M. (1969). Isolation of salmonellae and shigellae from an artificial mixture of fecal bacteria. Appl. Microbiol., 18: 4, 656-659.

Leifson, E. (1935). New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol., 40: 581-589.

Taylor, W.I., Harris, B. (1965) Isolation of shigellae. II. Comparison of plating media and enrichment broths. Am. J. Clin. Pathol. 44: 4, 476-479.

# *Sugar Free Agar*

**LAB087**

**Description**

A formula described by the International Dairy Federation for the enumeration of psychrotrophic and mesophilic Gram-negative rods in butter and other dairy products. The Gram-negative rods are able to deaminate proteins as a carbon source, whilst some enterococci are inhibited by this formula. The medium conforms to the formulation of the International Dairy Federation (I.D.F.).

| Typical Formula | g/litre |
|---|---|
| Gelatin Peptone | 7.5 |
| Tryptone | 7.5 |
| Sodium chloride | 5.0 |
| Agar No. 1 | 14.0 |

**Method for reconstitution**

Weigh 34 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, boil to dissolve and disperse into tubes or flasks. Sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Light straw, clear.

**pH:** 7.6 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation:** 0.2ml of butter fat in a pour plate technique.

**Incubation:** 30˚C for 2 days then 20˚C for a further two days – aerobically.

**Interpretation:** Count colonies.

**References**

International Dairy Federation (1964). International standard count of contaminating organisms in butter. International Standard FIL-IDF30.

Ritter, P. and Eschmann, K.H. (1966). Alimenta 5(2), 43-45.
Thomas, S. B. (1969). J. Appl. Bact. 32, 269-296.

Mossel, D.A.A., Krol, B. and Moerman, P.C. (1972). Alimenta 11(2), 51-60.

# *Susceptibility Test (Iso) Agar*

**LAB170**

**Description**

Susceptibility Testing 'Iso' Agar is a semi-defined medium for antimicrobial susceptibility (sensitivity) testing (AST), in which the undefined elements are maintained at minimum levels. The antimicrobial susceptibility test is utilised in epidemiological studies and in determining the appropriate usage of antimicrobials in the clinical environment. The response of clinical isolates to antimicrobials, and the detection of microbial resistance, allows for precise and rapid treatment. The AST is performed to detailed standards, the results of which must be reproducible; a major factor is the medium on which it is performed.

The presence of antagonists in the medium e.g. thymidine and metal ions, have a detrimental effect on results obtained. The addition of thymidine for the growth of dependant strains antagonises the antimicrobial action of Trimethoprim and sulphonamides and results in false resistance results. Metal ions can exert known antagonistic effects on a number of antibiotics. Therefore the anion and cation content of the medium must be regulated to prevent adverse effects on performance.

Susceptibility Testing 'Iso' Agar is produced having a stable mineral content, the presence of minimum antagonistic elements, and a constant isotonic pH (preventing the blocking or enhancement of antimicrobials), thereby ensuring production of optimum zones of microbial inhibition.

This medium will support the growth of the majority of pathogens requiring susceptibility testing, without the addition of supplements. However, certain organisms such as some streptococci, staphylococci, *Enterobacteriaceae* and *Neisseria* may require the addition of intrinsic growth factors e.g. lysed horse blood, thymidine, thiamine and menadione. However these supplements can introduce errors as they can affect the activity of certain antibiotics and consequently their affects must assessed before use.

| Typical Formula | g/litre |
|---|---|
| Peptone Mixture | 16.0 |
| Glucose | 2.0 |
| Starch | 1.0 |
| Sodium chloride | 2.8 |
| $Na_2HPO_4$ | 0.4 |
| Sodium glycerophosphate | 0.22 |
| Sodium gluconate | 0.1 |
| Sodium acetate | 1.0 |
| Uridine | 0.3 |
| Defined Chemical Mixture | 0.078 |
| Agar | 12.0 |

**Method for Reconstitution**

Weigh 35.9 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and if required add 5-7% sterile lysed horse blood. Pour into sterile petri dishes and allow to set. Cool to 47°C, mix well and dispense into petri dishes.

**Appearance:** Straw clear gel.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms:
(as recommended by the British Society for Antimicrobial Chemotherapy (BSAC))

*Escherichia coli* NCTC 10418
*Staphylococcus aureus* NCTC 6571
*Pseudomonas aeruginosa* NCTC 10662
*Enterococcus faecalis* NCTC 29212
*Haemophilus influenzae* NCTC 11931
*Streptococcus pneumoniae* ATCC 49619

**Storage:** Capped containers - up to 3 months at 15-20°C in the dark. Plates - up to 7 days at 2-8°C, in the dark.

**Inoculation:** Surface, inoculum as described by standard methods.

**Incubation:** As stipulated in the BSAC methodology.

**References**

Ericsson, H.M., Sherris, J.C. (1971). Antibiotic Sensitivity Testing. Report of an International Collaborative Study. Acta. Pathol. Microbiol. Scand. Sect B Suppl.; **217**: 1-90.

Amato, R.F., Thornberry, C., (1979). Calcium and Magnesium in Mueller Hinton Agar and their influence on disc diffusion susceptibility results. Current Microbiol. **2**: 135-138.

Hawkey, P.M., Birkenhead, D., Kerr, K.G., Newton, K.E., Hyde, W.A. (1993). Effect of divalent cations in bacteriological media on the susceptibility of Xanthomonas maltophila to imipenem with special reference to zinc ions. J. Antimicrobial Chermother; **31**: 181-183.

Garrod, L.P., Waterworth, P.M. (1969). Effect of medium composition on the apparent sensitivity of pseudomonas aeruginosa to gentimicin. J. Clin. Pathol; **22**: 534-538.

Duncan, I.B.R. (1974). Susceptibility of 1500 isolates of Pseudomonas aeruginosa to gentimicin, carbenecillin, colistin, and polymyxin B. Antimicrobial Agents and Chermother; (Jan) 9-15.

# *T.C.B.S. Cholera Medium*

***(Thiosulphate Citrate Bile Salts Sucrose Agar)***

**LAB096**

**Description**

T.C.B.S. is designed for the selective isolation of *Vibrio* species, particularly *V. cholerae.* The formulation was developed by Kobayashi, Enomoto, Sakazaki and Kuwahara and inhibits most of the *Enterobacteriaceae* for at least 24 hours. Therefore heavy inoculation of the medium is possible.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 5.5 |
| Peptone Mix | 10.0 |
| Sodium thiosulphate | 10.0 |
| Sodium citrate | 10.0 |
| Bile salts | 9.0 |
| Sucrose | 17.0 |
| Sodium chloride | 10.0 |
| Ferric citrate | 1.0 |
| Bromothymol blue | 0.04 |
| Thymol blue | 0.04 |
| Agar No. 1 | 15.0 |

**Method of reconstitution**

Weigh 88 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil. Cool to 47˚C and pour into Petri dishes. DO NOT AUTOCLAVE OR OVERHEAT THIS MEDIUM.

**Appearance:** Dark green clear agar.

**pH:** 8.6 ± 0.2

Minimum Q.C. organisms: *V. cholerae* WDCM 00136
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 4˚C in the dark. Capped containers – up to 1 month at 15-20˚C in the dark.

**Inoculation:** Surface plating with a heavy inoculum, streak out to single colonies.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Vibrio. cholerae* | 2.0-3.0 | CV.E.G. | Yellow | may revert to green at R.T. |
| *V. parahaemolyticus* | 3.0-5.0 | CV.E.G. | Blue or Green | |
| Enterococci | 1.0 | CV.E.G. | Yellow | |
| *Proteus* spp. | 1.0 | F.CR.G. | Green/ Yellow | |

**References**

Kobayashi, T., Enomoto, S., Sakazaki, R. and Kuwahara, S. (1963). Jap. Bacteriol 18: 10-11, 387-391.

# *Tetrathionate Broth Base A.P.H.A.*

**LAB097**

**Description**

A selective enrichment broth for the growth of *Salmonella typhi* and other *Salmonella* spp. from faeces, foods etc. It conforms to the formulation recommended by the American Public Health Association for use in the examination of dairy products and foods for salmonellae. Organisms which reduce tetrathionate, such as salmonellae, proliferate in the medium, whilst most enteric organisms are inhibited. Certain members of the *Proteus* group will also reduce tetrathionate thereby impairing the performance of the medium in some cases. To overcome this, Novobiocin may be added to the medium at a level of 40 microgram/ml before addition of the iodine. Gram-positive organisms are inhibited by the inclusion of bile salts.

| Typical Formula | g/litre |
|---|---|
| Balanced Peptone No. 1 | 5.0 |
| Bile Salts | 1.0 |
| Calcium carbonate | 10.0 |
| Sodium thiosulphate | 30.0 |

**Method for reconstitution**

Weigh 46 grams of powder and add to 1 litre of deionised water. Bring to the boil with frequent swirling to fully dissolve the medium. Cool to 45˚C and add 20ml of iodine solution prepared as indicated below. Mix well before dispensing into bottles and continue swirling whilst dispensing to avoid the calcium carbonate sedimenting. For the best results the medium should be used the same day as prepared.

**Iodine solution:** Dissolve 5g of potassium iodide and 6g of iodine crystals in 20ml of distilled water.

**Appearance:** Turbid white.

**pH:** 8.4 ± 0.2

Minimum Q.C. organisms: *Salmonella* sp. WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 7 days at 4˚C in the dark (without iodine solution).

**Inoculation:** Add 1 part of sample suspension or inoculated pre-enrichment medium to 9 parts of Tetrathionate Broth.

**Incubation:** 12-24 hours at 37˚C.

**Subculture:** Onto LAB034 Brilliant Green Agar and either LAB032 XLD or LAB110 Hektoen Enteric or other *Salmonella* selective media.

**References**

Standard methods for the Examination of Dairy products, 10th Edition. APHA, (1953).

## *Thioglycollate Medium (Brewer)*

**LAB064**

**Description**

This is the original formula introduced by Brewer in 1940 as a clear medium for the cultivation of anaerobes. It has found applications as a sterility test medium and as a blood culture medium although it has been superseded by Fluid Thioglycollate LAB025 and Fastidious Anaerobe Broth LAB071 for these purposes.

The agar makes the medium viscous slowing down the permeation of oxygen and any convection currents. Sodium thioglycollate acts as a reducing agent and also neutralises the bacteriostatic properties of mercurial compounds. Methylene blue is a redox indicator which is colourless at low Eh but turns green on exposure to oxygen.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 1.0 |
| Yeast Extract | 2.0 |
| Balanced Peptone No. 1 | 5.0 |
| Dextrose | 5.0 |
| Sodium chloride | 5.0 |
| Sodium thioglycollate | 1.1 |
| Methylene blue | 0.002 |
| Agar No. 1 | 1.0 |

**Method for reconstitution**

Weigh 20 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes then bring to the boil with gentle agitation to dissolve the solids. Distribute into screw top containers leaving minimal headspace. Sterilise by autoclaving at 121˚C for 15 minutes. Tighten caps as soon as possible after autoclaving.

**Appearance:** Straw coloured, translucent, viscous liquid which may have a green surface due to contact with oxygen. If the medium has a diffuse green tinge it should not be used until the oxygen has been driven off by holding in a boiling water bath for 5 minutes. Do not reheat more than once.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *C. perfringens* WDCM 00007

**Storage of Prepared Medium:** Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Ensure adequate dispersal of the inoculum in the broth.

**Incubation:** 37˚C for 24-72 hours.

**Growth Indicators:** A diffuse turbidity or individual colonies.

**References**

Brewer, J.H. (1940). Clear liquid mediums for the culture of anaerobes. J. Amer. Med. Ass. 115: 598-600.

## *Todd Hewitt Broth*

**LAB075**

**Description**

A nutritious broth medium formulated by Todd and Hewitt for the production of antigenic streptococcal haemolysin. Todd Hewitt Broth is also used to cultivate streptococci prior to serological grouping. The use of a fermentable sugar in the formulation leads to the production of acid which would normally inactivate the haemolysin. This is prevented by the inclusion of buffers to maintain the pH of the medium thus preserving the haemolysin, as well as promoting the growth of pneumococci.

| Typical Formula | g/litre |
|---|---|
| Infusion from fat-free minced meat | 10.0 |
| Tryptone | 20.0 |
| Dextrose | 2.0 |
| Sodium bicarbonate | 2.0 |
| Sodium chloride | 2.0 |
| Disodium phosphate anhydrous | 0.4 |

**Method for Reconstitution**

Weight 36.4grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and warm to dissolve. Dispense into 10ml volumes in screw capped containers and sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Pale straw, clear broth.

**pH:** 7.8 ± 0.2

**Inoculation:** Pick a well isolated colony for subculture into Todd Hewitt Broth.

**Incubation:** 37˚C for 18-48hrs, aerobically.

**Storage:** Capped containers - up to 3 months at 15-20ºC in the dark.

Minimum Q.C. Organisms *Streptococcus pyogenes* ATCC 19615.

**Reference:**

Todd, E.W., and Hewitt, L.F., (1932) A New Culture Medium for the Production of Antigenic Streptococcal Haemolysin. J. Path. Bact. 35 (1) 973-974.

Updyke, E.,L., and Nickle, M.I. (1954) A Dehydrated Medium for the Preparation of Type Specific Extracts of Group A Streptococci. Appl. Microbiol. 2 117-118.

## *Triple Sugar Iron Agar*

**LAB053**

**Description**

This is a modification of the Krumwiede and Kohn medium of 1917 which differentiates some of the *Enterobacteriaceae* on the basis of four reactions; fermentation of lactose, glucose and sucrose and $H_2S$ production. This medium should be used in conjunction with a urease test to eliminate *Proteus* spp. when screening for *Salmonella* spp.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 3.0 |
| Yeast Extract | 3.0 |
| Balanced Peptone No. 1 | 20.0 |
| Sodium chloride | 5.0 |
| Lactose | 10.0 |
| Sucrose | 10.0 |
| Glucose | 1.0 |
| Ferric citrate | 0.3 |
| Sodium thiosulphate | 0.3 |
| Phenol red | 0.025 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 65 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil with frequent swirling to dissolve the solids. Distribute into tubes and sterilise at 121˚C for 15 minutes. Allow to set as a slope ensuring that the slant is over a butt approximately 3cm deep.

**Appearance:** Reddish-orange gel.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** A heavy inoculum is streaked over the surface of the slope and stabbed into the butt.

**Incubation:** 37˚C aerobically for 24 hours.

**Interpretation**

| Slant/butt | Colour | Utilisation |
|---|---|---|
| Alkaline/acid | Red/yellow | Glucose only fermented Peptones utilised |
| Acid/acid | Yellow/yellow | Glucose fermented Lactose + or sucrose fermented |
| Alkaline/alkaline | Red/Red | Neither glucose, lactose, nor sucrose fermented Peptones utilised |

| Organism | Butt | Slant | Sulphide production |
|---|---|---|---|
| *S. sonnei* | Acid | or | - |
| *S. flexneri* | | Alk. | |
| *Salmonella typhi* | Acid | NC | + |
| *S. typhimurium* | Acid | | |
| *S. enteritidis* | Gas | NC | + |
| *E. coli* | | | |
| *Enterobacter aerogenes* | Acid Gas | Acid | - |
| *Proteus mirabilis* | Acid Gas | Acid | + |

NC = No Change

**References**

American Public Health Association (1963). Diagnostic Procedures and Reagents, 4th edn., A.P.H.A., New York.

American Public Health Association (1966). Recommended Methods for the Microbiological Examination of Foods. 2nd edn., A.P.H.A., New York.

Edwards, P.R. and Ewing, W.H. (1962). Identification of *Enterobacteriaceae.* Burgess Publishing Co., Minneapolis.

# *Tryptone Bile Agar*

**LAB072**

**Description**

First introduced by Delaney, McCarthy and Grasso in 1962 as a method for detecting faecal coliforms in water supplies based on the production of indole on a bile medium at 44˚C. The idea was applied to foodstuffs by Anderson and Baird-Parker in 1975. The inoculum is placed onto the membrane on a resuscitation agar and incubated at 37˚C for 4 hours. The membrane is then transferred to a Tryptone Bile Agar plate and incubated at 44˚C: after incubation the membrane is flooded with indole reagent. Indole positive colonies produce a red colour on the membrane and are easily counted.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Bile Salts No. 3 | 1.5 |
| Agar No. 2 | 15.0 |

**Method for reconstitution**

Weigh 36.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Cool to 47˚C and pour into Petri dishes.

**Appearance:** Straw coloured, clear gel.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 4 days at 2-8˚C in the dark. Capped container – up to 1 month at 2-8˚C in the dark.

**Inoculation:** 1ml of a 1:10 dilution of homogenised sample onto a membrane. The recommended membranes are 85mm in diameter with a 0.45 micron pore size manufactured from cellulose esters.

**Incubation:** 4 hours at 37˚C on Nutrient Agar LAB008 or Minerals Modified Glutamate Medium LAB080A and 80B plus agar – then 18-24 hours on Tryptone Bile Agar at 44˚C.

**Indole reagent:** 5% p-dimethylaminobenzaldehyde in N-HC1 (Vracko & Sherris 1963).

**Indole reaction:** Pipette 1-2ml of reagent into Petri dish lid, remove membrane with forceps and place on reagent. Allow to stand for 5 minutes for reaction to develop, then dry in sunlight to 'fix' the colour. Count all pink colonies as *E. coli*.

**Growth characteristics**

| organism | colony size (mm) | shape & surface | indole reaction on membrane |
|---|---|---|---|
| *E. coli* | 1.0-3.0 | CV.E.G. | positive – pink colour |
| other *Enterobacteriaceae* | 0.5-2.0 | CV.E.G. | negative – no colour |
| *Pseudomonas* spp. | no growth | | |
| *Staphylococcus* spp. | no growth | | |
| *Bacillus* spp. | no growth | | |

**References**

Anderson, J.M., Baird-Parker, A.C. (1975). A rapid and direct method for enumerating Eschericia coli biotype I in food. J. Appl. Bact. 39: 111-117.

Delaney, J.E., McCarthy, J.A. & Grasso, R.J. (1962). Measurement of *E. coli* type I by the membrane filter technique Wat. Sewage Wks. 109, 289.

Baird, R.M., Corry, J.E.L., Curtis, G.D.U. (1988). Pharmacopoeia of culture media for food microbiology. Int. J. Food Microbiol. 276-277.

## *Tryptone Glucose Extract Agar*

**LAB063**

**Description**

A plate count agar suggested by the American Public Health Association (A.P.H.A.) for estimation of total viable counts in food and dairy products. This medium is also recommended by the Association of Official Analytical Chemists (A.O.A.C.).

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 3.0 |
| Tryptone | 5.0 |
| Glucose | 1.0 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 24 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then boil to dissolve before distributing into tubes or bottles. Sterilise at 121˚C for 15 minutes.

**Appearance:** Pale straw colour, clear.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00032
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Pour plate technique.

**Incubation:** 30˚C aerobically for 48 hours for aerobic mesophile count. 6˚C aerobically for 10 days for aerobic psychrotroph count. 55˚C aerobically for 48 hours for aerobic thermophile count.

**References**

Association of Official Analytical Chemists (AOAC). (1995). Bacteriological Analytical Manual, 8th ed.: Association of Official Analytical Chemists.

Hausler, W.J. (Ed.) (1976). Standard Methods for the Examination of Dairy Products, 14th edn.: American Public Health Association.

Speck, M.J. (Ed.) (1976). Compendium of Methods for the Microbiological Examination of Foods.: American Public Health Association.

## *Tryptone Soy Agar*

***(Soybean Casein Digest Medium)***

**LAB011**

**Description**

A general purpose agar which will support the growth of a wide range of micro organisms. The performance of this product conforms with that laid down by the Harmonised Pharmacopeia (USP/EP/JP) for sterility testing. The medium can be used for phage typing, colicine typing and for testing the X and V factor requirements of *Haemophilus* spp.

| Typical Formula | g/litre |
|---|---|
| Tryptone (Casein Digest USP) | 15.0 |
| Soy Peptone | 5.0 |
| Sodium chloride | 5.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 37 grams of powder, disperse in 1 litre of deionised water, allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121˚C. Cool to 47˚C, mix well and then pour plates.

**Appearance:** Pale straw coloured, clear gel.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* ATCC 6538
*Pseudomonas aeruginosa* ATCC 9027
*Bacillus subtilis* ATCC 6633
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC 16404

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Surface plating.

**Incubation:** Time and temperature to suit organisms, usually aerobic.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *S. aureus* | 1.0-1.5 | CV.E.G. | White-Yellow | |
| *Ps. aeruginosa* | 0.5-3.0 | F.CR.D. | Grey-Green | (marked strain variation) |

**References**

Harmonised Pharmacopeia 8.0, volume 1.

Blair, J.E. and Carr, M. (1953). The bacteriophage typing of staphylococci. J. Infect. Dis. 93: 1-13.

Examination of Dairy Products. A.P.H.A., New York.

## *Tryptone Soy Broth*

***(Soybean Casein Digest Medium***

**LAB004**

**Description**

A general purpose nutritious broth capable of growing a wide range of bacteria and fungi. This medium is formulated to and performs to the requirements of the Harmonised Pharmacopeia for the sterility testing of a wide range of pharmaceutical products. The medium is also widely used for blood cultures although the high carbohydrate level may cause rapid growth and subsequent death of acid-producing organisms.

| Typical Formula | g/litre |
|---|---|
| Tryptone (Casein Digest USP) | 17.0 |
| Soy Peptone | 3.0 |
| Sodium chloride | 5.0 |
| Dipotassium phosphate | 2.5 |
| Dextrose | 2.5 |

**Method for reconstitution**

Weigh 30 grams of powder, disperse in 1 litre of deionised water. Swirl to mix and warm if necessary to dissolve. Dispense into tubes or flasks and sterilise at 121˚C for 15 minutes. Do not exceed temperature.

**Appearance:** Straw coloured, clear.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms:
*Bacillus subtilis* ATCC 6633
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC16404

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Incubation:** 20-25˚C aerobically for 14 days, for sterility tests. 37˚C aerobically for 14 days for blood cultures.

**Growth indicators:** Turbidity or precipitate.

**References**

Harmonised Pharmacopeia 8.0, volume 1.

## *Tryptone Soy Broth*

***(without dextrose)***

**LAB205**

**Description**

A basal media that can be supplemented with carbohydrates and indicators for fermentation studies.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 17.0 |
| Soy Peptone | 3.0 |
| Sodium chloride | 5.0 |
| Dipotassium phosphate | 2.5 |

**Method for reconstitution**

Weigh 27.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and warm to dissolve if necessary. Distribute into tubes or bottles and sterilise by autoclaving for 15 minutes at 121°C. Do not exceed stated temperature.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: straw, clear liquid

**pH:** 7.3 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Staphylococcus aureus* WDCM 00032
*Bacillus subtilis* WDCM 00003
*Candida albicans* WDCM 00054
*Aspergillus niger* WDCM 00053

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight
Prepared media: capped containers – up to 3 months at 15-20°C in the dark.

**Incubation:**

Aerobically at 20-25°C for 14 days, for sterility tests.
Aerobically at 37°C for 14 days for blood cultures.

## *Tryptone Water*

**LAB129**

**Description**

A substrate for the testing of an organism's ability to produce indole from tryptophan. The indole test is frequently used in the classification of coliform organisms. This product is preferable to peptone water LAB104 because it has a higher content of tryptophan.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Sodium chloride | 5.0 |

**Method for reconstitution**

Weigh 15 grams of powder, disperse in 1 litre of deionised water. Heat to dissolve then distribute into screw cap bottles. Sterilise by autoclaving at 121˚C for 15 minutes.

**Appearance:** Colourless, clear.

**pH:** 7.5 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** From pure culture.

**Incubation:** 37˚C for 24-48 hours.

**Interpretation:** Indole positive organisms will give a distinct colour change when either Kovac's or Ehrlich's indole reagent is added.

**References**

American Public Health Association. (1955). American Water Works Association 10th edn. 391-392.

MacFaddin, J. (1983). Biochemical tests for the identification of medical bacteria. 2nd edn. Williams & Wilkins, Baltimore.

## *Tryptose Phosphate Broth*

**LAB062**

**Description**

This is a versatile, nutritionally rich buffered glucose broth. The medium is a general purpose broth that has been used as a blood culture medium, and with the addition of sodium azide 0.025% as a selective medium for streptococci in dairy products.

| Typical Formula | g/litre |
|---|---|
| Tryptose | 20.0 |
| Dextrose | 2.0 |
| Sodium chloride | 5.0 |
| Disodium phosphate | 2.5 |

**Method for reconstitution**

Weigh 29.5 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, heat to dissolve solids then distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, pale straw liquid

Minimum Q.C. organisms: *E. coli* WDCM 00013
*S. aureus* WDCM 00034
*Strep. pyogenes* NCTC 8198

**pH:** 7.3 ± 0.2

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20˚C in the dark.

**Inoculation:** For blood culture work dilute sample at least 1:10 in broth.

**Incubation:** Dependent on application.

**References**

American Public Health Association (1948). Standard Method for the Examination of Dairy Products, 10th edn. A.P.H.A., New York.

American Public Health Association, (1950). Diagnostic Procedures and Reagents, 3rd edn., A.P.H.A., New York.

# *T.Y.C. Medium*

***(Tryptone Yeast Cystine)***

**LAB035**

**Description**

A medium designed by J. D. de Stoppelaar in 1967 to differentiate *Streptococcus sanguis* (frequently found in dental plaque) from *Streptococcus mutans* (implicated in dental caries). The medium uses a high sucrose content to promote the formation of specific glucans by *S. mutans* thus forming distinctive colonies. It can be made selective by the addition of 0.2 units per ml of Bacitracin.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 15.0 |
| Yeast Extract | 5.0 |
| L-Cystine | 0.2 |
| Sodium sulphite | 0.1 |
| Sodium chloride | 1.0 |
| Disodium phosphate anhydrous | 0.8 |
| Sodium bicarbonate | 2.0 |
| Sodium acetate anhydrous | 12.0 |
| Sucrose | 50.0 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 98 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise for 15 minutes at 121˚C. Cool to 47˚C, mix and pour plates.

**Appearance:** White, translucent gel.

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms: *S. mutans* NCTC 10832

**Storage of Prepared Medium:** Plates – up to 7 days at 4˚C in the dark.

**Inoculation:** Surface, streaking out for single colonies.

**Incubation:** 37˚C for 4-5 days in an atmosphere of 90% $H_2$, 10% $CO_2$.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Strep. mutans* Type A | 1.0-3.0 | 'heaped' colony granular surface irregular edge | Yellow | Crumbles when touched with wire |
| Type B | 1.0-3.0 | As Type A | Grey white | soft consistency, white precip in agar (glistening drop) |
| *S. sanguis* | 1.0-3.0 | Convex glossy crenated | White | very rubbery (glistening drop) |

**References**


de Stoppelaar, J.D. van de Houte, J. and de Moor, C.E. (1967). The presence of dextran forming bacteria resembling *Streptococcus bovis* and *Streptococcus sanguis* in dental plaque. Arch. Oral Biol. 12: 1199-1201.

de Stoppelaar, J.D. (1971). *Streptococcus mutans, Streptococcus sanguis* and dental caries. Thesis, Rijksuniversiteit, Utrecht.

Emilson, C.G., Bratthall, D. (1976). Growth of *Streptococcus mutans* on various selective media. Journal of Clinical Microbiology 4: 95-98.

Gold, O.G., Jordon, H.V., Van Houte, J. (1973). A selective medium for *Streptococcus mutans*. Archives of Oral Biology 19: 1357-1364.

Ikeda, T., Sandham, H.J. (1972). A high-sucrose medium for the identification of *Streptococcus mutans*. Archives of Oral Biol. 4: 781-783.

Wade, W.G., Alldred, M. J., Walker, D.M. (1986). J. Med. Microbiol. 22: 319-323. An improved medium for isolation of *Streptococcus mutans*.


# *Urea Agar Base*

***(Christensen)***

**LAB130**

**Description**

This is a modification of Christensen's urea base for the detection of rapid urease production by *Proteus* spp. Other enterobacteria will split the urea, but this will be delayed. This delay is achieved by the incorporation of glucose and the introduction of a buffering system into the medium. The indicator for ammonia production is phenol red.

| Typical Formula | g/litre |
|---|---|
| Peptone | 1.0 |
| Glucose | 1.0 |
| Sodium chloride | 5.0 |
| Disodium phosphate | 1.2 |
| Potassium dihydrogen phosphate | 0.8 |
| Phenol red | 0.012 |
| Agar No. 1 | 12.0 |

**Method for reconstitution**

Weigh 2.1 grams of powder, disperse in 95ml of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise at 121˚C for 15 minutes. Allow to cool to 47˚C, add aseptically 5ml sterile urea solution X130/X135. Distribute into sterile bottles and slopes, allow to set in the sloped position.

**Appearance:** Yellow/pale pink, translucent.

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms:
*Proteus mirabilis* ATCC 29906/WDCM 00023
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped container – up to 1 month at 2-8˚C in the dark.

**Inoculation:** Pure culture using straight wire for stab/streak technique.

**Incubation:** 37˚C for 4-6 hours or overnight, aerobically.

**Interpretation:** Production of red colour in under 6 hours is positive for rapid urease production.

**Organism Growth Characteristics**

| | | |
|---|---|---|
| *Proteus* spp. | Red colour | 4-6 hours |
| *Klebsiella* spp. | Red colour | 18-24 hours |
| *Staphylococcus* spp. | Red colour | 24-48 Hours |

**References**


Christensen, W.B. (1946). Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. J. Bacteriol. 52: 461-466.

# *Urea Broth Base*

***(Christensen)***

**LAB131**

**Description**

This is a liquid version of Christensen's medium (LAB130) introduced by Maslen in 1952. This modification allows inoculation by Pasteur pipette, and it is easier to detect contamination in a fluid rather than in a slope. Maslen also claimed that it is easier to detect positive results.

| Typical Formula | g/litre |
|---|---|
| Peptone | 1.0 |
| Glucose | 1.0 |
| Disodium phosphate | 1.2 |
| Potassium dihydrogen phosphate | 0.8 |
| Sodium chloride | 5.0 |
| Phenol red | 0.004 |

**Method for reconstitution**

Weigh 0.9 grams of powder, add to 95ml of deionised water. Swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. Allow to cool to 47˚C then add aseptically 5ml of X130/X135 sterile urea solution. Distribute into sterile screw cap bijou bottles.

**Appearance:** Yellow, clear.

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms:
*Proteus mirabilis* ATCC 29906/WDCM 00023
*E. coli* WDCM 00013

**Storage of Prepared Medium:** Capped container – up to 1 month at 2-8˚C in the dark.

**Inoculation:** Fluid culture by pasteur pipette or straight wire from pure growth.

**Incubation:** 37˚C for 4-6 hours – preferably in a water bath for most rapid growth, aerobically.

**Interpretation:** The production of a red colour in under six hours is a positive result for rapid urease.

**Organism Growth Characteristics**

| | | | |
|---|---|---|---|
| *Proteus* spp. | | Red colour | 4-6 hours |
| Some strains | *Klebsiella* | Red colour | 18-24 hours |
| | *Escherichia* | ,, | ,, |
| | *Staphylococcus,* | ,, | ,, |

**References**

Maslen L.G.C. (1952). Routine use of liquid urea medium for identifying Salmonella and Shigella organisms. J. Brit. Med. 2: 545-546.

# *UVM Base*

**LAB155**

**Description**

UVM (University of Vermont Medium) Base is a two stage selective enrichment broth for the isolation of *Listeria* from meat products and environmental swabs. The original method has been modified to replace the second stage broth (UVM II) with Fraser broth LAB164 (McClain & Lee 1989).

| Typical Formula | g/litre |
|---|---|
| Tryptone | 5.0 |
| Meat Peptone | 5.0 |
| Beef Extract | 5.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 20.0 |
| Disodium hydrogen phosphate | 9.6 |
| Potassium dihydrogen phosphate | 1.35 |
| Aesculin | 1.0 |

**pH:** 7.4 ± 0.2

**Appearance:** Straw opalescent broth

**Method for reconstitution**

Weigh 52 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise at 121˚C for 15 minutes. Cool to 47˚C and add 2 vials of UVM I supplement (X155) as required. Mix well and distribute into sterile tubes or bottles.

**Inoculation:** Add 25g sample to 225ml of and homogenise.

**Incubation:** 30˚C aerobically for 24 hrs. Subculture onto selective agars. .

Minimum QC organism:
*Listeria monocytoges* WDCM 00020
*E. coli* (inhibition) WDCM 00013

**References**

McClain D., and Lee W.H. (1989) FSIS method for isolation of L.monocytogenes from processed meat and poultry products. Lab.Comm.No.57, Revised May 24, 1989. US Dept of Agric.FSIS, Microbiol. Div.

Warburton D.W. *et al* (1991) A Canadian comparative study of modified versions of the FDA and USDA methods for the detection of L.monocytogenes. J.Food Protection 54 (9) 669-676.

# *Violet Red Bile Agar*

***(V.R.B.A.)***

**LAB031**

**Description**

A medium for the enumeration of coliform organisms in food and dairy products. The selectivity of the medium is due to the presence of bile salts and crystal violet. Lactose fermenters produce red/purple colonies often surrounded by a halo of the same colour. Non lactose fermenters produce pale colonies. Selectivity can be increased by incubation at 42-44°C.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 3.0 |
| Balanced Peptone No. 1 | 7.0 |
| Sodium chloride | 5.0 |
| Bile Salts No. 3 | 1.5 |
| Lactose | 10.0 |
| Neutral red | 0.03 |
| Crystal violet | 0.002 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Disperse 38.5g of powder in 1 litre of distilled water. Dissolve by bringing to the boil with frequent swirling of the flask to prevent overheating. DO NOT AUTOCLAVE. Cool to 45°C and distribute into bottles or tubes. If held molten in a water bath, use within 3 hours.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: light, purple-violet clear gel

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*Enterococcus faecalis* (inhibition) WDCM 00087

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 1 month at 15-20˚C in the dark.

**Inoculation:** Pour plate (with or without overlay) or surface spread.

**Incubation:** 37˚C for 18-24 hours for 'coliforms'; 4˚C for 10 days for psychrotrophs; 32˚C for 24-48 hours for mesotrophs; 42˚C for 18 hours for thermotrophs.

**Interpretation:** Count all red/purple colonies > 0.5mm in diameter. Calculate the number of coliforms in original sample.

**References**


American Public Health Association (1972), Standard Methods for the Examination of Dairy Products. 13th edn. (ed. W.H. Hausler), A.P.H.A., Washington.

American Public Health Association (1966). Recommended Methods for the Microbiological Examination of Foods. 2nd edn. (ed. J.M. Sharf) A.P.H.A., Washington.

Davis, J.G. (1951). Milk Testing Dairy Industries, London.

Mossel, D.A.A., Eelderink, I. and Sutherland, J.P. (1977). Development and use of single, 'polytropic' diagnostic tubes for the approximate taxonomic grouping of bacteria, isolated from foods, water and medicinal preparations. Zbl. Bakt. Hyg. I., Orig., A 278, 66-79.

Mossel, D.A.A., Eelderink, I., Koopmans, M. and van Rossem, F. (1979). Influence of carbon source, bile salts and incubation temperature on the recovery of *Enterobacteriaceae* from foods using MacConkey type agars. J. Food Protec. 42, 470-475.

Mossel, D.A.A., van der Zee, H., Hardon, A.P. and van Netten, P. (1986). The enumeration of thermotropic types amongst the *Enterobacteriaceae* colonizing perishable foods. J. Appl. Bacteriol. 60, 289-295.


# *Violet Red Bile Agar with MUG*

***(V.R.B.A. with MUG)***

**LAB573**

**Description**

Violet Red Bile Agar with MUG (Methylumbelliferyl-β-D-glucuronide) is a medium for the simultaneous enumeration of coliform organisms and *Escherichia coli* in food and dairy products. The selectivity of the medium is due to the presence of bile salts and crystal violet. Lactose fermenters produce red/purple colonies often surrounded by a halo of bile precipitate. *Escherichia coli* produce red/purple fluorescent colonies due to the fermentation of lactose and production of the enzyme glucuronidase, which hydrolyses MUG to yield the fluorescent compound methylumbelliferone, detectable by long-wave UV light. Non-lactose fermenters produce pale colonies.

Standard Methods procedures specify VRBA with MUG for detecting *E. coli* in food and dairy products by fluorescence.

| Typical Formula | g/litre |
|---|---|
| Yeast extract | 3.0 |
| Balanced peptone No.1 | 7.0 |
| Sodium chloride | 5.0 |
| Bile Salts No. 3 | 1.5 |
| Lactose | 10.0 |
| Neutral red | 0.03 |
| Crystal violet | 0.002 |
| Agar No. 2 | 12.0 |
| MUG, 4 methylumbelliferyl-ß-D-glucuronide | 0.1 |

**Method for reconstitution**

Weigh 38.6 grams of powder, disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and then sterilise the medium, with frequent mixing, by bringing to the boil. DO NOT AUTOCLAVE. Cool to 47°C and distribute into bottles or tubes. If held molten in a water bath, use within 3 hours.

**Appearance:** Light purple-violet clear gel.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Enterobacter aerogenes* WDCM 000175
*Staphylococcus aureus* WDCM 00034

**Storage of Prepared Medium:** Plates - up to 7 days at 2 - 8°C in the dark. Capped containers up to 1 month at 15-20°C in the dark.

**Inoculation:** Pour plate method (with or without overlay) or surface spread.

**Incubation:** 37°C for 18-24 hours for 'coliforms'; 4°C for 10 days for psychrotrophs; 32°C for 24-48 hours for mesotrophs; 42°C for 18 hours for thermotrophs.

**Interpretation:** Examine plates for growth and fluorescence. Count all red/purple colonies > 0.5mm in diameter as coliforms and all fluorescent colonies as presumptive *E. coli*. Calculate the number of coliforms and *E. coli* in the original sample.

**References**


Christen, G.L., Davidson, P.M., McAllistair, J.S. and Roth, L.A. (1993). Coliform and other indicator bacteria. 247-269. R.T. Marshall (ed.) Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Hitchens, A.D., Hartman, P.A. and Todd, E.C.D. (1992) Coliforms-*Escherichia coli* and its toxins. C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Hitchens, A.D., Peng, P., Watkins, W.D., Rippey, S.R. and Chandler, L.A. (1995). *Escherichia coli* and the coliform bacteria. 4.01-4.29. Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.

Feng, P.C.S. and Hartman, P.A. (1982). Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. 43:1320-1329.

Chang, G.W., Brill, J. and Lum, R. (1989). Proportion of β-D-glucuronidase negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. 55:335-339.

Hansen, W. and Yourassowsky, E. (1984). Detection of β-D-glucuronidase in lactose fermenting members of the family Enterobacteriaceae and its presence in bacterial urine cultures. J. Clinical Microbiol. 20:1177-1179.

Kilian, M. and Bulow, P. (1976). Rapid diagnosis of Enterobacteriaceae. Acta. Pathol. Microbiol. Scand. Sect. B. 84:245-251.

Mates, A. and Shaffer, M. (1989). Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Microbiol. 67:343-346.

Damare, J.M., Campbell, D.F. and Johnston, R.W. (1985). Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. Journal of Food Science. 50:1736-1746.

## *Violet Red Bile Glucose Agar*

***(V.R.B.G.A.)***

**LAB088**

### Description

A modification of Violet Red Bile Agar LAB031 introduced by Mossel in 1978. V.R.B.A. LAB031 contains lactose which is fermented by members of the coli/aerogenes group, this medium gives a 'coliform' count. V.R.B.G.A. LAB088 has substituted lactose with glucose. Glucose is fermented by all members of the *Enterobacteriaceae* thus V.R.B.G.A. gives a presumptive *Enterobacteriaceae* count. Bile salts and crystal violet are used to inhibit Gram positive and non-enteric organisms. The overlay procedure ensures anaerobic conditions and suppresses the growth of non-fermentative Gram negative bacteria.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 3.0 |
| Balanced Peptone No. 1 | 7.0 |
| Sodium chloride | 5.0 |
| Bile Salts No. 3 | 1.5 |
| Glucose | 10.0 |
| Neutral red | 0.03 |
| Crystal violet | 0.002 |
| Agar No. 2 | 12.0 |

### Method for reconstitution

Weigh 38.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Bring to the boil with frequent swirling to prevent overheating. Further sterilisation is not required. Cool to 45˚C, mix well and dispense into tubes or bottles. If held molten in a water bath, use within 3 hours.

**Appearance:** Light purple-violet, clear.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms:
*E. coli* WDCM 00013
*Enterococcus faecalis WDCM 00087* (inhibition)

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped containers – up to 1 month at 15-20˚C in the dark.

**Inoculation:** Pour plate method with overlay.

**Incubation:** 37˚C aerobically for 18-24 hours.

**Interpretation:** Count all red/purple colonies > 0.5mm in diameter. Calculate the number of *Enterobacteriaceae* in original sample.

### References

Pharmacopoeia of Culture Medium for Food Microbiology (1987). Int. J. Food Microbiol. 5: 3: 280-81.

Mossel, D.A.A., Mengerink, W.H.J. and Scholts, H.H. (1962). Use of a modified MacConkey agar medium for the selective growth and enumeration of *Enterobacteriacaea.* J. Bacteriol. 84: 381.

## *Water Plate Count Agar (ISO)*

**LAB197**

### Description

A nutritious non-selective medium which conforms to **ISO 6222:1999(E)** *Water quality - Enumeration of culturable micro-organisms - Colony count by inoculation in a nutrient agar culture medium.* The estimation of overall numbers of microorganisms can be used for the assessment and surveillance of water quality. Colony counts are useful for assessment of ground water integrity and the efficiency of water treatment processes. They also give an indication of the cleanliness and integrity of the distribution system. They can be used to assess the suitability of a water supply for the preparation of food and drink, thus avoiding contamination of the product with spoilage organisms. The main value of colony counts lies in the detection of changes in water supply quality from those expected, based on frequent long term monitoring. A sudden increase in the numbers can be a warning of pollution and can call for immediate remedial action

| Typical Formula | g/litre |
|---|---|
| Tryptone | 6.0 |
| Yeast Extract | 3.0 |
| Agar | 15.0 |

### Method for reconstitution

Weigh 24.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix then sterilise at 121°C for 15 minutes. Cool to 47°C before use.

**Appearance:** Pale straw coloured, clear gel.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* WDCM 00032
*Escherichia coli* WDCM 00013

**Storage of Prepared Medium:** Plates can be stored up to 7 days at 2-8°C in the dark.

**Inoculation:** Pour plate technique or surface inoculation.

**Incubation:** Aerobically at 36°C ± 2°C for 44 ± 4 hours and 22°C ± 2°C for 68 ± 4 hours.

**Interpretation:** Count all colonies and calculate the number of organisms (or 'colony forming units' c.f.u.) per ml of sample allowing for dilution factors.

### References

ISO 6222 (1999) Water quality - Enumeration of culturable microorganisms - Colony count by inoculation in a nutrient agar medium.

# *W. L. Nutrient Agar*

***(Wallerstein Laboratory)***

**LAB079**

**Description**

This medium was developed by Green and Gray in 1950 for the isolation and enumeration of yeasts, moulds and bacteria in the brewing process. The medium has a pH of 5.5 which is optimum for Brewers yeast and will allow the growth of a wide range of organisms including *Enterobacteriaceae*, *Flavobacterium*, *Lactobacillus* and *Pediococcus spp.* as well as yeasts and moulds. If a process involving bakers or distillers yeast is under examination the pH should be adjusted to 6.5. The medium may be adapted to detect bacteria only by the addition of 0.004 g/litre of Actidione to suppress the yeasts.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 4.0 |
| Tryptone | 5.0 |
| Dextrose | 50.0 |
| Potassium dihydrogen phosphate | 0.55 |
| Potassium chloride | 0.425 |
| Calcium chloride | 0.125 |
| Magnesium sulphate | 0.125 |
| Ferric chloride | 0.0025 |
| Manganese sulphate | 0.0025 |
| Bromocresol green | 0.022 |
| Agar No. 2 | 15.0 |

**Method for reconstitution**

Weigh 75 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. If adjustment of pH to 6.5 is required used 1% sodium bicarbonate. Cool to 47°C, mix well and dispense into Petri dishes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff
Finished medium: Green-blue, clear gel

**pH:** 5.5 ± 0.2

Minimum Q.C. organisms: *S. cerevisiae.* ATCC 9763

**Storage:**

Dehydrated culture media: 10-25°C

Final medium: up to 7 days (plates) or up to 1 month (capped containers) at 2-8°C in the dark

**Inoculation:** Surface plating or pour plate.

**Incubation:** 30˚C aerobically for 48 hours (bacteria); 20˚C aerobically for 48 hours (yeasts).

**Interpretation:** Count all colonies. Calculate organisms per ml in original sample.

**References**

Green, S.R. and Gray, P.P. (1950). Differential Procedure Applicable to Investigation in Brewing. Wallerstein Lab. Comm. 13,357.

Hall, J.F. (1971). Detection of Wild Yeasts in the Brewery. J. Inst. Brewing, 77: 513-516.

# *Wort Agar*

**LAB038**

**Description**

A medium for the enumeration of yeasts and moulds in butter. The medium can be modified to enable it to isolate osmophilic yeasts from soft drinks and sugar products by the addition of high concentrations of sucrose and glucose.

| Typical Formula | g/litre |
|---|---|
| Malt Extract | 15.0 |
| Yeast Extract | 7.0 |
| Sugars | 9.0 |
| Tartaric acid | 0.3 |
| Peptone | 1.0 |
| Dipotassium phosphate | 1.0 |
| Ammonium chloride | 1.0 |
| Agar | 14.0 |

**Method for reconstitution**

Weigh 48.3 grams of powder and disperse in 1 litre of deionised water. Add 2.35ml of glycerol. Allow to soak for 10 minutes, swirl to mix then sterilise for 15 minutes at 121°C. Use 60 grams per litre if required for inoculation by plate streaking with a wire loop. Do not exceed time or temperature of sterilisation. If osmophilic modification is required add 48.3 grams of powder to 1 litre of a solution containing 35% w/v sucrose and 10% w/v glucose then sterilise at 108˚C (5 p.s.i.) for 20 minutes.

**Appearance:** Light Brown, translucent.

**pH:** 5.0 ± 0.2

Minimum Q.C. organisms: *S. cerevisiae.* WDCM 00058

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 1 month at 15-20˚C in the dark.

**Inoculation:** Pour plate or surface spread.

**Incubation:** 25˚C aerobically for 5 days.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Candida* spp. | 4.0 | CV.E.G | White | |
| Fungi | Varies with species | | | |
| *S. cerevisiae* | 2.0-3.0 | Varies with strain | Cream | |

**References**

Parfitt, E.H. (1933). The influence of media upon the yeast and mould count of butter. J. Dairy Sci. 16: 141-147.

Scarr, M.P. (1959). Selective media used in the microbiological examination of sugar products. J. Sci. Fd. Agric. 10: 678-681.

## *Wort Broth (modified)*

**LAB099**

**Description**

A broth for the enumeration of yeasts and moulds, in butter. The medium can be modified for the isolation of osmophilic yeasts from soft drinks and sugar products by the addition of high concentrations of sucrose and glucose.

| Typical Formula | g/litre |
|---|---|
| Malt Extract | 15.0 |
| Peptone | 0.78 |
| Maltose | 12.75 |
| Dextrin | 2.75 |
| Dipotassium phosphate | 1.0 |
| Ammonium chloride | 1.0 |

**Method for reconstitution**

Weigh 33.3 grams of powder and disperse in 1 litre of deionised water, add 2.35ml of glycerol. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121˚C for 15 minutes. If osmophilic version is required disperse 33.3 grams of powder in 1 litre of a solution of 35% w/v sucrose and 10% w/v glucose then sterilise at 108˚C (5 p.s.i.) for 20 minutes.

**Appearance:** Light Brown, translucent.

**pH:** 4.8 ± 0.2

Minimum Q.C. organisms: *S. cerevisiae.* WDCM 00058

**Storage of Prepared Medium:** Capped container – up to 1 month at 15-20˚C in the dark.

**Incubation:** 25˚C aerobically for 5 days.

## *X.L.D. Agar*

***(Xylose Lysine Decarboxylase Agar)***

**LAB032**

**Description**

This medium was introduced by Taylor in 1965 to improve the recovery and recognition of *Shigella* spp, and has proved to be an excellent medium for *Salmonella* spp. The medium is low in nutrients and relies on a small amount of sodium desoxycholate for selectivity. The indicator system is novel and complex. Most enteric organisms except *Shigella,* will ferment xylose to produce acid. However the salmonellae will also decarboxylate the lysine to keep the pH neutral. At near neutral pH *Salmononella* can produce $H_2S$ from the reduction of thiosulphate producing black or black centred colonies. *Citrobacter* spp. can also decarboxylate lysine, however, the acid produced by fermentation of both lactose and sucrose will keep the pH too acid for $H_2S$ to be produced.

| Typical Formula | g/litre |
|---|---|
| Xylose | 3.75 |
| L-Lysine | 5.0 |
| Lactose | 7.5 |
| Sucrose | 7.5 |
| Sodium chloride | 5.0 |
| Yeast Extract | 3.0 |
| Phenol red | 0.08 |
| Agar No. 2 | 13.0 |
| Sodium desoxycholate | 1.0 |
| Sodium thiosulphate | 6.8 |
| Ferric ammonium citrate | 0.8 |

**Method for reconstitution**

Weigh 53.5 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix. Bring rapidly to the boil with frequent stirring, and transfer immediately to a 47˚C water bath. Pour into plates as soon as the medium has cooled. Protracted boiling or prolonged holding at elevated temperature induces precipitation.

**Appearance:** Light rose, clear gel.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *S.typhimurium* WDCM 00031
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking out for single colonies.

**Incubation:** 37˚C for 18-24 hours aerobically.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Salmonella* spp. | 1.0-2.5 | CV.E.G. | Trans. black centre | (clearing of acid ppt of coliforms) |
| *Shigella sonnei* | 1.5-2.5 | CV.E.G. | Pink | |
| *E. coli* | 0.5-1.5 | CV.E.G.(D) | Yellow | inhibited (ppt around colony) |
| *Citrobacter* spp. | 1.0-1.5 | CV.E.G.(D) | Yellow | (black centre) |
| *Proteus* spp | 1.0-2.5 | CV.E.G. | Trans. Pink (black centre) | fishy odour |

**References**


Taylor, W.I. (1965). Isolation of shigellae. I. Xylose Lysine Agars: New media for the isolation of enteric pathogens. Am. J. Clin. Pathol., 44: 471-475.

Taylor, W.I., and Harris, B. (1965). Isolation of shigellae. II. Comparison of plating media and enrichment broths. Am. J. Clin. Pathol., 44(4), 476-479.

Taylor, W.I., and Harris, B. (1967). Isolation of shigellae. III. Comparison of new and traditional media with stool specimens. Am. J. Clin. Pathol., 48: 350-355.

Taylor, W.I., and Schelhart, D. (1967). Isolation of shigellae. IV. Comparison of plating media with stools. Am. J. Clin. Pathol., 48: 356-362.

# *XLT4 Agar*

***Xylose Lysine Tergitol-4 Agar***

**LAB221**

### Description

XLT4 Agar is a selective differential isolation medium for the specific detection of *Salmonella* spp. from environmental, food and clinical samples. Due to its highly selective nature, XLT4 Agar is particularly effective when used with samples where overgrowth of contaminating flora is expected, for example, faecally-contaminated agricultural samples.

Developed to perform as per Miller & Tate in 1990, this medium was found to improve the recovery of non-typhi *Salmonella* from chicken and farm environmental samples. Dusch & Altwegg further established the application of XLT4 Agar to salmonellae detection in clinical samples, with the notable exceptions of *Salmonella* Typhi and *Salmonella* Paratyphi. The presence of peptone and yeast extract provides sufficient nutrients to allow the optimal growth of *Salmonella* spp.

Selectivity is provided by the anionic surfactant Niaproof® 4 (formerly known as Tergitol-4 / sodium tetradecylsulfate). This compound acts as an effective selective agent which is active against Gram-positive and many Gram-negative organisms, including *Proteus* spp.

Differentiation is based on fermentation of the sugars xylose, lactose and sucrose in addition to the decarboxylation of lysine. The inclusion of the pH indicator, phenol red, provides visual evidence of a pH decrease (yellow) or increase (red) in the medium. Ammonium iron (III) citrate is present to distinguish hydrogen-sulphide ($H_2S$) producing from non-$H_2S$ producing organisms.

Most enteric organisms, except *Shigella*, will ferment xylose to produce acid. However the salmonellae will also decarboxylate the lysine to keep the pH neutral to alkali, thus maintaining red colouration. At near-neutral pH *Salmonella* can produce $H_2S$ from the reduction of ammonium iron (III) citrate and thiosulphate ions producing black or black-centred colonies. Non $H_2S$-producing salmonellae will be red without a black centre.

Other Enterobacteriaceae (non-salmonellae) which are not inhibited by Niaproof-4, will ferment xylose, lactose and/or sucrose but will not decarboxylate lysine. This fermentation activity causes a decrease in pH, resulting in a colour change within the colonies from red to yellow.

| Typical Formula | g/litre |
|---|---|
| Proteose peptone | 12.00 |
| Yeast extract | 1.5 |
| L-lysine | 5.0 |
| Xylose | 3.5 |
| Lactose | 7.0 |
| Sucrose | 7.5 |
| Ammonium iron (III) citrate | 0.8 |
| Sodium thiosulphate | 5.5 |
| Sodium chloride | 5.0 |
| Phenol red | 0.08 |
| Agar | 13.0 |

### Method for reconstitution

Weigh 59 grams of powder and disperse in 1 litre of deionised water. Add 4.6ml of Niaproof-4 supplement (Sigma Niaproof® 4, product code N1404). Allow to soak for 10 minutes, swirl to mix. Heat the medium with frequent agitation and boil for 1 minute. DO NOT OVERHEAT OR AUTOCLAVE THIS MEDIUM. Cool to 48-50°C, mix well and dispense into sterile Petri dishes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff (may have a slight pink colouration)

Finished medium: clear, red gel

**pH:** 7.4 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:

*Salmonella Typhimurium* WDCM 0031
*Salmonella Enteritidis* WDCM 00030
*Escherichia coli* WDCM 00013
*Enterococcus faecalis* WDCM 00087

**Storage:**

Dehydrated culture media: 10-25°C.
Final medium: 7 days at 2-8°C in the dark

**Inoculation:** Surface inoculation, streaking out / spreading to achieve single colonies.

**Incubation:** Incubate plates at 37°C. Examine plates for growth at 24 hours and 48 hours.

**Interpretation:** Typical *Salmonella* (lactose-negative, $H_2S$ positive) appear as colourless or red colonies with a black centre, giving the traditional "fish-eye" appearance. All isolates with this appearance should be regarded as presumptive salmonellae.

Lactose-positive, $H_2S$ positive salmonellae, e.g. *Salmonella* Arizonae will appear yellow-red with black centre.

Lactose-negative, $H_2S$ negative salmonellae, e.g. *Salmonella* New Brunswick will appear yellow-red without a black centre.

Some strains of *Salmonella* Poona may demonstrate sensitivity to Niaproof® 4.

**Interpretation**

| organism | growth | colony size (mm) | shape & surface | $H_2S$ | colour |
|---|---|---|---|---|---|
| *Salmonella* Typhimurium | Good >50% recovery | 1.2 - 2.5 | CV, E, G | + | Clear/red, black centre |
| *Salmonella* Enteritidis | Good >50% recovery | 1.5 - 2.5 | CV, E, G | + | Clear/red, black centre |
| *Citrobacter freundii* | Good | 1.5 - 2.5 | CV/DR, E, D | +/- | Yellow |
| *Escherichia coli* | Suppressed | | | | |
| *Proteus mirabilis* | Inhibited | | | | |
| *Enterococcus faecalis* | Inhibited | | | | |
| *Staphylococcus aureus* | Inhibited | | | | |

**KEY** CV = Convex E = Entire D = Dull G = Glossy DR = Draughtsman

### References


Dusch, H. and Altwegg, M. (1995). Evaluation of five new plating media for the isolation of *Salmonella* species. *Journal of Clinical Microbiology*. **33**. No.4. 802-804.

Miller, R.G. and Tate, C.R. (1990). A highly selective plating medium for the isolation of *Salmonella*. *The Maryland Poultryman*, April: 2-7.

Miller, R.G., Tate, C.R., Mallinson, E.T. and Scherrer, J.A. (1991). Xylose-Lysine-Tergitol 4: An improved selective agar for the isolation of *Salmonella*. *Poultry Science* **70**. 2429-2432.

Miller, R.G., Tate, C.R., Mallinson, E.T. and Scherrer, J.A. (1992). *Erratum*. Xylose-Lysine-Tergitol 4: An improved selective agar for the isolation of *Salmonella*. *Poultry Science* **71**. 398.

Tate, C.R., Miller, R.G. and Mallinson, E.T. (1992). Evaluation of two isolation and non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag-swab samples. *J. Food Prot.* **55**. 964-967.

# *Yeast Extract Agar*

***(Yeastrel Milk Agar)***

**LAB018**

**Description**

A nutrient agar corresponding to the Standard Formulation for the plate count of micro-organisms in water and dairy products. This medium is also useful for teaching and demonstration purposes using non-fastidious organisms.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 3.0 |
| Balanced Peptone No. 1 | 5.0 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 23 grams of powder, disperse in 1 litre of deionised water. Free steam or boil to dissolve. Mix well, and dispense into containers. Sterilise for 15 minutes at 121˚C.

To prepare Yeastrel Milk Agar add 10ml of fresh milk before autoclaving.

**Appearance:** Pale straw, clear gel.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms: *E. coli* WDCM 00013
*S. aureus* WDCM 00032

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark. Capped container – up to 3 months at 15-20˚C in the dark.

**Inoculation:** Pour plate technique or surface spreading.

**Incubation:** 30˚C aerobically for 48 hours for aerobic mesophile count. 6˚C aerobically for 10 days for aerobic psychrotroph count. 55˚C aerobically for 48 hours for aerobic thermophile count.

**References**

Environment Agency: The Microbiology of Drinking Water (2002). Methods for the Examination of Water and Associated Materials.

British Standard 4285: Methods of Microbiological Examination for Dairy Purposes.

# *Yeast Extract Dextrose Chloramphenicol Agar*

**LAB119**

**Description**

A selective medium for the enumeration of yeasts and moulds in milk and other dairy products. The medium is said to have superior storage properties to O.G.Y.E. and also has the advantage of incorporating an autoclavable supplement.

| Typical Formula | g/litre |
|---|---|
| Yeast Extract | 5.0 |
| Dextrose | 20.0 |
| Agar No. 1 | 15.0 |

**Method for reconstitution**

Weigh 40 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to boil. Add 2 vials of X009 (X209) which have been dissolved in ethanol and autoclave at 121˚C for 10 minutes. Allow to cool to 45˚C before using with poured plate technique. THIS MEDIUM MUST NOT BE RE-AUTOCLAVED.

**Appearance:** Pale yellow, clear.

**pH:** 6.6 ± 0.2

Minimum Q.C. organisms:
*Aspergillus* sp. WDCM 00053
*S. cerevisiae* WDCM 00058
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Incubation:** 25˚C for 5 days, aerobically.

**Inoculation:** Pour plate technique.

**Interpretation:** Count all colonies.

**References**

Engel, G. (1982). Verglich verschieden Nährböden zum quantitativen Nachweis von Hefen und Schimmelpilzen in Milch und Milchprodukten. Milchwiss. 37: 727-730.

International Organisation for Standardization (ISO): Milk and milk products – enumeration of yeasts and moulds – colony count technique at 25˚C – standard method ISO/DIS 6611.

International Milchwirtschaftsverband: Milch und Milchprodukten – Zählung von Hefen und Schimmelpilzen (Kolonieählung bel 25 C). – International IMV Standard 94: (1980) in Milchwiss. 36: 220-222.

Normenausschufl Lebensmittel und landwirtshaft. Produkte in DIN Deutsches Institut für Normung e.V. Mikrobiologische Milchuntersuchung. Bestimmung der Anzahl von Hefen und Schimmelpilzen. Reference method DIN 10186.

British Standards Institute. B.S. 4285. Section 3.6: (1986).

# *Yeast & Mould Agar*

**LAB200**

**Description**

A medium recommended for the isolation and maintenance of yeasts and moulds. This medium can also be used for the detection of wild yeasts in beer.

| Typical Formula | g/litre |
|---|---|
| Yeast extract | 3.0 |
| Malt extract | 3.0 |
| Peptone | 5.0 |
| Dextrose | 10.0 |
| Agar | 20.0 |

**Method for reconstitution**

Weigh 41.0 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C, mix well and dispense into sterile Petri dishes.

Increased selectivity of the medium can be achieved by the addition of 12-15ml of sterile lactic acid (X037) to reduce the pH to 4.0. For the detection of wild yeasts, supplement the medium to the desired level with copper sulphate prior to sterilisation.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear straw gel

**pH:** 6.2 ± 0.2

**Hazard classification**

NR – Not regulated

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Candida albicans* WDCM 00054
*Saccharomyces pastorianus* NCYC 185
*Lactobacillus fermetum* ATCC 9338

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight
Poured plates: 7 days at 2-8°C in the dark

# *Yersinia Selective Agar*

***(Schiemann's C.I.N. Agar)***

**LAB120**

**Description**

This medium is based on the work of Schiemann. It is used for the isolation and enumeration of *Yersinia* spp. from clinical samples and from food. The selective components are sodium desoxycholate, crystal violet, cefsulodin, irgasan and novobiocin. Yersiniae ferment mannitol with an intense, localised, acid production in the centre of the colony which produces a red 'bull's eye' appearance. The ratio of transparent border to red centre varies with serotype and environmental strains may appear rough with an irregular edge. Most other enteric bacteria, if they grow, produce a larger colony with a diffuse pinkish centre and opaque outer zone.

| **Typical Formula** | **g/litre** |
|---|---|
| Peptone Mixture | 22.5 |
| Mannitol | 20.0 |
| Sodium chloride | 1.0 |
| Magnesium sulphate | 0.01 |
| Sodium pyruvate | 2.0 |
| Sodium desoxycholate | 0.5 |
| Neutral red | 0.03 |
| Crystal violet | 0.001 |
| Agar No. 2 | 12.0 |

**Method for reconstitution**

Weigh 58 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, then bring to the boil for 1 minute only. DO NOT AUTOCLAVE. Allow to cool to 47˚C add 2 ampoules C.I.N. supplement X120. Mix well, pour plates.

**Appearance:** Red, clear.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms: *Y. enterocolitica* WDCM 00038
*E. coli* (inhibition) WDCM 00013

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8˚C in the dark.

**Inoculation:** Surface, streaking out for single colonies.

**Incubation:** 30˚C aerobically for 24 hours.

**Growth Characteristics**

| **organism** | **colony size (mm)** | **shape & surface** | **colour** | **other** |
|---|---|---|---|---|
| *Y. enterocolitica* | 1.0-2.5 | CV.E.G. | Red centre | Colony varies with strain, may be rough & irregular |
| Gram +ve organisms | no growth | | | |

**References**


Schiemann, D.A. (1979). Synthesis of a selective agar medium for *Yersinia enterocolitica.* Can. J. Microbiol. 25: 1298-1304.

Schiemann, D.A. (1982). Development of a two step enrichment procedure for recovery of *Yersinia enterocolitica* from food. Appl. Eniviron. Microbiol. 43: 14-27.

Mossel, D.A.A. (1987). Cefsulodin Irgasan Novobiocin (C.I.N.) agar. Int. J. Food. Microbiol. 5: 208, 209.

# 2. Harlequin™ Chromogenic Media

The Harlequin™ chromogenic microbiological culture media range has been developed to improve the isolation and identification of a range of microorganisms. Traditional culture media generally rely on the fermentation of sugars or other biochemical reactions for presumptive identification of bacteria. Harlequin™ chromogenic media provide a more specific identification by detection of specific enzymes produced by certain groups of bacteria. The advantage of this type of media is that it can eliminate or reduce the need for subculture and the performance of confirmatory biochemical tests to determine the identity of some microorganisms.

Chromogenic substrates act as the substrate for specific enzymes and change colour due to the action of the enzyme. Lab M have their own patented chromogenic compounds called the novel CHE (cyclohexenoesculetin) substrates which give the bacterial colony a black non-diffusing colouration when hydrolysed by the enzyme involved in the presence of iron salts. Some of the CHE derivatives are used in Harlequin™ media along with indolyl derivatives (5-bromo-4-choro-3-indolyl), which give a blue-green colour on cleavage.

Chromogenic media are most rapidly gaining acceptance as an indicator for *Escherichia coli*. The β-D-glucuronide enzyme is present in approximately 95% of *E. coli* and is uncommon in the other *Enterobacteriaceae*. This type of test is now used widely for water and food microbiology. There is also an increase in interest for chromogenic *Salmonella* media as traditional tests have a very poor specificity resulting in many false positive results. The use of chromogenic media simplifies *Salmonella* testing and saves much time in unnecessary confirmation tests.

## Harlequin™ Salmonella ABC

**HAL001**

**Description**

*Salmonella* spp. can be differentiated from other members of the family *Enterobacteriaceae* by their ability to produce α-galactosidase in the absence of β-galactosidase. This medium, developed for the isolation of *Salmonella* spp. from food and clinical samples, utilises a dual chromogen system to visualise these enzyme activities. This medium will also detect *Salmonella typhi* and *paratyphi.*

The first substrate, CHE-β-Gal, is enzymatically cleaved by β-galactosidase producing organisms giving black colonies in the presence of iron. Most *Enterobacteriaceae* are β-galactosidase positive and these produce black colonies on *Salmonella* ABC. The second substrate, X-α-Gal, is hydrolysed by *Salmonella* spp. producing green colonies that are easily distinguished from the black or colourless colonies of other organisms. The medium is based on D.C.A Hynes and hence utilises sodium desoxycholate and sodium citrate as inhibitors. Isolation of *Salmonella* spp. by culture remains the most reliable method of detection. However, most media are highly non-specific and consequently place a heavy burden on the laboratory in terms of biochemical and serological confirmation of suspect colonies. With improved specificity, the ABC medium dramatically reduces the need for 'false positive' screening, saving labour and reducing consumable costs.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 5.0 |
| Peptone | 5.0 |
| Sodium citrate | 8.5 |
| Sodium desoxycholate | 5.0 |
| Agar | 12.0 |
| X-α-Gal | 0.08 |
| CHE-β-Gal | 0.3 |
| Ferric ammonium citrate | 0.5 |
| IPTG | 0.03 |

**Method for reconstitution**

Weigh 36.5 grams of powder, disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and then sterilise the medium by bringing to the boil. Cool to 47°C, mix well and dispense into Petri dishes.

**DO NOT REMELT OR AUTOCLAVE THIS MEDIUM.**

**Appearance:** Translucent straw gel.

**pH:** 7.2 ± 0.2

Minimum QC organisms:
*Salmonella typhimurium* WDCM00031
*Escherichia coli* WDCM 00013

**Storage of Prepared Medium:** Plates - up to 7 days at 2 - 8°C in the dark.

**Inoculation:**
**Clinical:** Streak for single colonies after selective enrichment in Selenite Broth.
**Food:** Streak for single colonies after selective enrichment.

**Incubation:** 37°C aerobically for 18 - 24 hours

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Salmonella* spp. | 1.5 - 3.5 | CV.E.G. | Green | (Black if β-galactosidase +ve) |
| *Shigella* spp. | 3.0 - 4.0 | CV.E.G. | Colourless | (Black if β-galactosidase +ve) |
| *E.coli* | PP - 2.5 | CV.E.G. | Black | (No Growth) |
| *Proteus* spp. | PP - 1.0 | CV.E.G. | Colourless | (Fishy Odour) |

**References**

Perry, J.D., Ford, M., Taylor, J., Jones, A., Freeman, R., Gould, F.K., (1999). ABC Medium, a New Chromogenic Agar for Selective Isolation of *Salmonella* spp. J. Clin. Micro. 37: 766-768.

## Harlequin™ TBGA (TBX)

***(Tryptone Bile Glucuronide Agar)***

**HAL003**

**Description**

A medium developed for the simple enumeration of *E. coli* without the need for membranes, or pre-incubation on Minerals Modified Glutamate Medium. It is based upon the formulation of Tryptone Bile Agar, LAB072, the medium has been modified by the addition of a chromogenic substrate to detect the ß-glucuronidase enzyme, which is highly specific for *E. coli**, and is detected by the MUG reagent in other formulations. The advantage of the chromogenic substrate is that it requires no UV lamp to visualise the reaction, and it is concentrated within the colony, facilitating easier enumeration in the presence of other organisms, or when large numbers are present on the plate.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Bile Salts No.3 | 1.5 |
| X-glucuronide | 0.075 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 36.5 grams of powder, disperse in 1 litre of deionised water and allow the mixture to soak for 10 minutes. Swirl to mix and sterilise at 121°C for 15 minutes. Cool to 47°C and pour in to Petri dishes. Dry the surface prior to inoculation.

**Appearance:** Straw, clear gel.

**pH:** 7.2 ± 0.2

Minimum QC organisms: *Escherichia coli* WDCM 00013 (blue/green)

**Inoculation:** Inoculate 0.5 ml of a 1:10 dilution of the sample and spread over the entire surface of the plate. Further dilution may be necessary if large numbers of *E. coli* are present, to ensure colonies can be easily counted.

**Incubation:** 30°C for 4 hours, followed by 18 hours at 44°C.

**Interpretation:** Count all blue/green colonies as presumptive *E. coli*, calculate the cfu/g in the original material. A simple indole test can be performed by placing one drop of Kovac's reagent onto a colony and if positive, a red halo will appear in the medium around the colony. If negative, then the halo will be white.

*96-97% of *E. coli* strains positive. A notable exception is *E. coli* 0157:H7.

### References


Dibb, W.L. and Bottolfsen, K.L. (1984). Evaluation of Rosco Diagnostic ß-glucuronidase Tablets in the Identification of Urinary Isolates of *Escherichia coli*. Acta Path.Microbiol. Immunol. Scand. Sect. B 92 261-264.

Hansen, W. and Yourassowsky, E. (1984). Detection of ß-glucuronidase in Lactose Fermenting Members of the Family Enterobacteriaceae and its Presence in Bacterial Urine Cultures. J. Clin. Micro.20 (6) 1177-1179.

Robinson, B.J. (1984). Evaluation of a Fluorogenic Assay for Detection of *E. coli*. App & Env. Microbiol.48 (2) 285-288.

Perez, J.L., Berrocal, C.I. and Berrocal, L. (1986). Evaluation of a Commercial ß-glucuronidase Test for the Rapid and Economical Identification of *Escherichia coli*. J.App.Bacteriol. 61 541-545.

Raghubeer, E. and Matches, J.R. (1990). Temperature Range for Growth of *Escherichia coli* Serotype 0157:H7 and Selected Coliforms in *E. coli* Medium. J.Clin. Micro. 28 (4) 803-805.

Bolton, F.J. (1995) Personal Communication


## *Harlequin™ LB Agar*

**HAL004**

**Refer to the Biomolecular Section**

## *Harlequin™ SMAC-BCIG*

***(Sorbitol MacConkey Agar with BCIG)***

**HAL006**

### Description

This is a specific substrate medium for the isolation of *Escherichia coli* O157:H7, the primary serovar associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Pathogenicity of the organism is linked to the production of verocytotoxins (VT1 and VT2), but it should be noted that not all strains of O157 produce verocytotoxins, and that strains from other serovars can be toxin producers (e.g. O26, O103, O111, O113, O145). *E. coli* O157 has been associated epidemiologically with food poisoning outbreaks involving beef burgers and cold cooked meats.

This medium is a modification of Sorbitol MacConkey Agar (SMAC). The addition of the chromogenic substrate BCIG (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide) improves the specificty of the medium. *E. coli* O157:H7 is typically sorbitol negative and β-glucuronidase negative producing pale translucent colonies on this medium. The majority of other *E. coli* strains are β-glucuronidase positive and sorbitol positive (pink/red colonies). A small percentage of *E. coli* are β-glucuronidase positive and sorbitol negative and thus appear as blue/green colonies on this medium. Consequently this medium can distinguish between non-O157 sorbitol negative *E. coli* and the genuine toxigenic *E. coli* O157:H7. This reduces the number of unnecessary confirmation tests that are performed. The medium can be made more selective by the addition of Cefixime Tellurite supplement X161 to prepare CT-SMAC. Most workers recommend the use of CT-supplemented medium alongside unsupplemented medium to ensure maximum isolation of *E. coli* O157. This medium can also be useful for the detection of other VTEC producing *E. coli* in conjunction with specifically targetted IMS particles (***Captivate™***).

| **Typical Formula** | **g/litre** |
|---|---|
| Peptone | 20.0 |
| Sorbitol | 10.0 |
| Bile Salts No. 3 | 1.5 |
| Sodium Chloride | 5.0 |
| BCIG | 0.1 |
| Neutral Red | 0.03 |
| Crystal Violet | 0.001 |
| Agar | 12.0 |

### Method for reconstitution

Weigh 48.6 grams of powder and add to 1 litre of de-ionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C, add 2 vials of X161 CT supplement and pour plates. Dry the surface prior to inoculation.

**Appearance:** Pale red, light violet tinge.

**pH:** 7.1 ± 0.2

Minimum QC organisms:
*Escherichia coli O157:H7* WDCM 00014 (non-toxigenic)
*Escherichia coli* WDCM00013
*Enterococcus faecalis* WDCM 00084 (inhibition)

**Inoculation:** From O157 Broth LAB 165, surface streak for single colonies.

**Incubation:** 37°C aerobically for 18-24 hr. Examine plates for sorbitol negative, β-glucuronide negative colonies. Confirm as O157:H7 by serology, (commercial kits or antiserum available).

### Growth Characteristics

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *E. coli* O157:H7 sorbitol -ve β-glucuronide -ve | 2.5 - 4.0 | CV.E.G. | Translucent |
| *E. coli* sorbitol +ve β-glucuronide +ve | 2.5 - 4.0 | CV.E.G. | Pink/red or purple centre |
| *E. coli* sorbitol -ve β-glucuronide +ve | 2.5 - 5.0 | CV.E.G. | Green or translucent with green centre |

*Note:* Sorbitol positive toxigenic *E. coli* O157:H7 have been isolated and appear as sorbitol positive and β-glucuronide positive on this medium.

### References


1) Okrend, A.J.G., Rose, B.E., and Lattuada, C.P. (1990) Use of 5-Bromo-4-Chloro-3-Indoxyl-*β*-D-Glucuronide in MacConkey Sorbitol Agar to Aid in the Isolation of *Escherichia coli* O157:H7 from Ground Beef. J.Food Protection **53** (11) 941-943

# *Harlequin™ E. coli/Coliform Medium*

**HAL008**

**Description**

This dual chromogenic substrate medium has been developed for the simultaneous enumeration of *Escherichia coli* and coliforms in food and environmental samples. The different colony types are simple to distinguish allowing rapid counting of both *E. coli* and coliforms on a single medium.

Based upon the formulation of Tryptone Bile Agar LAB072, the medium has been modified by the addition of two chromogenic substrates, one to detect the ß-glucuronidase enzyme (X-glucuronide) and another to detect the ß-galactosidase enzyme (magenta-ß-gal). Typical *E. coli* strains possess both enzymes but only cleave the X-glucuronide substrate, thereby producing blue-green colonies. Typical coliforms, however, possess only the ß-galactosidase enzyme and produce rose-pink colonies.

The colony types are easily distinguishable, even in the presence of other organisms, or when large numbers are observed, making simultaneous enumeration of *E. coli* and coliforms a quick and simple procedure.

N.B. This product is not available for sale in the USA.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Bile Salts No.3 | 1.5 |
| X-glucuronide | 0.075 |
| Magenta-β-galactoside | 0.1 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 36.6 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw gel.

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Enterobacter aerogenes* WDCM 00175
*Staphylococcus aureus* WDCM 00034 (Inhibition)

**Storage of Prepared Medium:** Plates - up to 7 days at 2 - 8°C in the dark.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Escherichia coli* | 1.0 - 2.0 | CV.E.G. | Blue-Green | |
| *Enterobacter aerogenes* | 1.5 - 2.5 | CV.E.G. | Rose-Pink | |
| *Pseudomonas aeruginosa* | 0.5 - 1.0 | F.CR.D/ CV.E.G. | Buff | |
| *Enterococcus faecalis* | | | | No growth |
| *Staphylococcus aureus* | | | | No growth |

**Inoculation:** Inoculate 0.5 ml of a 1:10 dilution of the sample and spread over the entire surface of the plate. Further dilution may be necessary if large numbers of *E. coli* and/or coliforms are present, to ensure colonies can be easily counted.

**Incubation:** 18 - 24 hours at 37° C

**Interpretation:** Count all blue-green colonies as presumptive *E. coli*, and calculate the cfu/g. Count all rose-pink colonies as presumptive coliforms, and calculate cfu/g.

**References**

1) Baylis, C.L., Patrick, M. (1999). Comparison of a range of Chromogenic media for enumeration of total Coliforms and *Escherichia coli* in foods. Leatherhead International Technical Notes. No.135: 99.

# *Harlequin™ mLGA*

***(Membrane Lactose Glucuronide Agar)***

**HAL009**

**Description**

Traditionally, membrane Lauryl Sulphate Broth (mLSB) has been used as the standard media for isolating coliforms (including *E. coli*) from water potentially contaminated with sewage. Harlequin™ membrane Lactose Glucuronide Agar (mLGA) is a modification of mLSB aimed at reducing costs by reducing the number of filters used per test sample and aiding in the recovery and identification of coliforms and *E. coli*. The medium has been modified from the mLSB formulation by the incorporation of X-glucuronide, sodium pyruvate and agar. X-glucuronide is incorporated to allow for the presumptive isolation of *E. coli*, sodium pyruvate aids the recovery of chlorine stressed organisms and agar is incorporated to remove the need for absorbent pads. This medium is recommended for the enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique in The Microbiology of Drinking Water 2002 (previously Report 71).

| Typical Formula | g/litre |
|---|---|
| Peptone | 39.0 |
| Yeast Extract | 6.0 |
| Lactose | 30.0 |
| Phenol Red | 0.2 |
| Sodium Lauryl Sulphate | 1.0 |
| Sodium Pyruvate | 0.5 |
| X-Glucuronide | 0.2 |
| Agar | 10.0 |

**Method for reconstitution**

Disperse 88 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise by autoclaving at 115°C for 10 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:** Red, clear gel.

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Enterobacter aerogenes* WDCM 00175
*Staphylococcus aureus* WDCM 00034 (inhibition)

**Storage of Prepared Medium:** Plates - up to 7 days at 2-8°C in the dark.

**Inoculation:** *E. coli* and coliform counts can be performed on the same sample of water. The volume and dilution of test sample should be chosen so as the number of colonies on the membrane lies between 20 and 80. With waters expected to contain low numbers of coliforms, a sample of 100ml should be filtered. For full methodology refer to The Microbiology of Drinking Water 2002 section 4 B - The enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique.

**Incubation:** 4 hours at 30 °C followed by 14 hours at 37 °C

**Interpretation:** Count all green-blue colonies as presumptive *E. coli*, and all green-blue and yellow colonies as presumptive coliforms.

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Escherichia coli** | 0.5 - 1.5 | CV.E.G. | Green | Yellow if glucuronidase -ve |
| Lactose fermenters | 0.5 - 1.5 | CV.E.G. | Yellow | |
| Non-lactose fermenters | 0.5 - 1.5 | CV.E.G. | Red | |
| *Staphylococcus aureus* | | | | No growth (suppressed) |

*96-97% of E. coli strains positive. A notable exception is E. coli O157:H7

**References**

Sartory, D.P. & Howard, L. (1992). A medium detecting B-glucuronidase for the simultaneous membrane filtration enumeration of *Escherichia coli* and coliforms from drinking water. *Letters in Applied Microbiology* **15**, 273-276.

Calabrese, J.P. & Bisssonette, G.K. (1990). Improved membrane filtration method incorporating catalase and sodium pyruvate for detection of chlorine stressed coliform bacteria. *Applied and Environmental Microbiology* **56**, 3558-3564.

Microbiology of Drinking Water 2002 section 4 B - Environment Agency. The enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique.

# *Harlequin™ Listeria Chromogenic Agar (ISO)*

**HAL010**

**Description**

Listeria Chromogenic Agar (according to the formulation of Ottaviani and Agosti) is a selective medium for the isolation and presumptive identification of *Listeria monocytogenes* from foodstuffs and related materials as described in ISO 11290-1:1997.

Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Cycloheximide provide the medium's selectivity. Chromogenic activity is as a result of a chromogenic substrate for the detection of the β-glucosidase enzyme, common to all *Listeria* spp. and to a few strains of Enterococci and Bacilli.

The specific differential activity of this agar is obtained with a proprietary lecithin substrate for the detection of the phospholipase enzyme that will only be present in the *L. monocytogenes* colonies growing on this media. This enzyme activity will result in a halo of precipitation surrounding the target colonies.

With the combination of both the chromogenic and phospholipase enzyme reactions, it is possible to differentiate *Listeria monocytogenes* (blue colonies surrounded by an opaque halo) from other *Listeria spp* (blue colonies without an opaque halo).

| Typical Formula | g/litre |
|---|---|
| Meat Peptone | 18.0 |
| Tryptone | 6.0 |
| Yeast extract | 10.0 |
| Lithium chloride | 10.0 |
| Sodium chloride | 5.0 |
| Disodium hydrogen orthophosphate anhydrous | 2.5 |
| Sodium pyruvate | 2.0 |
| Glucose | 2.0 |
| Glycerophosphate | 1.0 |
| Magnesium sulphate | 0.5 |
| 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside | 0.05 |
| Agar | 13.5 |

**Method for reconstitution**

Weigh 70.5 grams of powder and disperse in 950mL of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 48-50°C, and add 2 vials of reconstituted X072 supplement. Swirl to mix. Add 2 vials of X010 supplement (**pre-heated to 48-50°C**). Mix well with gentle end-over-end mixing and dispense into Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: opaque, cream-yellow gel

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Listeria monocytogenes* WDCM 00021
*Escherichia coli* WDCM 00013 (Inhibited)

**Storage:**

Dehydrated culture media: 10-25°C

Final medium: 7 days at 2-8°C in the dark

**Inoculation:** Surface inoculation - streak out to single colonies. This medium is highly selective and a heavy inoculum can be used.

**Incubation:** 37°C aerobically for 48 hours.

**Interpretation**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *Listeria monocytogenes* | 1 - 2 | Round, Regular | Blue to blue-green, surrounded by opaque halo |
| *Listeria* spp. | 1 - 2 | Round, Regular | Blue to blue-green, without opaque halo |

Isolates presumptively identified as *Listeria* spp. and *Listeria monocytogenes* must be subjected to further biochemical tests to confirm their identity. Some strains of *Listeria ivanovii* may demonstrate lecithinase activity.

**References**

ISO 11290-1:1997 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Listeria monocytogenes* - Part 1: Detection method. Incorporating Amendment 1.

# *Harlequin™ CSIM (ISO)*

***Harlequin™ Cronobacter sakazakii Isolation Medium (ISO)***

**HAL012**

**Description**

*Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) is a member of the *Enterobacteriaceae* family and has been associated with serious outbreak infections in neonates (premature infants) which have been fed on infant formula milk. Although rarely causing infections in immunocompetent adults, *C. sakazakii* has been implicated in sepsis, meningitis and necrotising enterocolitis with a high death rate in neonates. This opportunistic pathogen is common in the environment and its ability to survive desiccation presents a significant risk for post pasteurisation contamination and survival in spray dried milk products.

*C. sakazakii* appears to constitutively express high levels of α-glucosidase. This enzyme hydrolyses the chromogenic substrate 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside present in the medium, producing green to blue-green coloured colonies. Other *Enterobacteriaceae* such as *E. coli* do not express strong α-glucosidase activity and appear colourless or purple due to the uptake of crystal violet.

The combination of sodium desoxycholate, crystal violet and elevated incubation temperature produce a very selective and specific medium. Non-*Enterobacteriaceae* may appear colourless or violet coloured (due to their inability to hydrolyse the chromogenic substrate) or are inhibited by the selective components and incubation temperature.
This media formulation is currently recommended as part of the isolation protocol under ISO/TS 22964:2006(E) for the isolation of *Enterobacter sakazakii* from milk and milk products.

| Typical Formula | g/litre |
|---|---|
| Pancreatic peptone of casein | 7.0 |
| Yeast extract | 3.0 |
| Sodium chloride | 5.0 |
| Sodium desoxycholate | 0.6 |
| 5-bromo-4-chloro-3-indolyl-α-D-Glucoside | 0.15 |
| Crystal violet | 0.002 |
| Agar | 14.0 |

**Method for reconstitution**

Weigh 29.75 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, purple gel

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms:
*Cronobacter sakazakii* ATCC 12868
*Enterobacter aerogenes* WDCM 00175
*Bacillus cereus* WDCM 00001
*Staphylococcus aureus* WDCM 00034

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** Following selective enrichment in Modified Lauryl Sulphate Tryptose Broth Vancomycin Medium, streak onto HAL012 Harlequin™ *Cronobacter sakazakii* Isolation Medium (ISO).

**Incubation:** Incubate at 44°C + 0.5°C for 24 hours + 2 hours.

**Interpretation:** After incubation the plate should be assessed for typical colonies of *C. sakazakii*. Typical colonies are 1-3mm and are green to blue-green.

**References**


Bowen AB, Braden CR (2006). "Invasive *Enterobacter sakazakii* disease in infants". *Emerging Infect Dis* **12** (8): 1185–9.

Caubilla-Barron J & Forsythe S (2007). "Dry stress and survival time of *Enterobacter sakazakii* and other Enterobacteriaceae in dehydrated infant formula". *Journal Food Protection* **13**: 467-472.

"*Enterobacter sakazakii* infections associated with the use of powdered infant formula--Tennessee, 2001" (2002). *MMWR Morb Mortal Wkly Rep* **51** (14): 297–300.

Farmer JJ III, Asbury MA, Hickman FW, Brenner DJ, the Enterobacteriaceae Study Group (USA) (1980). "*Enterobacter sakazakii*: a new species of "Enterobacteriaceae" isolated from clinical specimens". *Int J Syst Bacteriol* **30**: 569–84.

ISO/TS 22964:2006(E) Milk and milk products – Detection of *Enterobacter sakazakii*.

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# *Harlequin™ Cronobacter sakazakii Agar – DFI Formulation*

***(Druggan, Forsythe & Iversen)***

**HAL013**

**Description**

*Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) is a member of the *Enterobacteriaceae* family and has been associated with serious outbreak infections in neonates (premature infants) which have been fed on infant formula milk. Although rarely causing infections in immunocompetent adults, *C. sakazakii* has been implicated in sepsis, meningitis and necrotising enterocolitis with a high death rate in neonates. This opportunistic pathogen is common in the environment and its ability to survive desiccation presents a significant risk for post pasteurisation contamination and survival in spray dried milk products.

Based on the formulation described by Druggan, Forsythe and Iversen, Harlequin™ *Cronobacter sakazakii* Agar is a medium on which *Cronobacter sakazakii* appears to constitutively express high levels of α-glucosidase. This enzyme hydrolyses the chromogenic substrate 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside present in the medium, producing green coloured colonies. Other *Enterobacteriaceae* such as *E. coli* do not express strong α-glucosidase activity and appear colourless. Hydrogen sulphide producing organisms, such as Salmonella, and *Proteus* spp. appear grey, brown or black on this formulation due to the production of precipitated ferrous sulphate, which results from the hydrogen sulphide produced by these organisms interacting with ferric ions in the medium. This reaction prevents the weakly α-glucosidase positive *Proteus vulgaris* from appearing as green on the medium.

Selectivity is achieved from the inclusion of sodium desoxycholate which serves to inhibit the growth of most Gram-positive organisms.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 15.0 |
| Soya peptone | 5.0 |
| Sodium chloride | 5.0 |
| Ferric ammonium citrate | 1.0 |
| Sodium desoxycholate | 1.0 |
| Sodium thiosulphate | 1.0 |
| X-α-D-glucopyranoside | 0.1 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 43.1 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw-coloured gel

**pH:** 7.3 ± 0.2

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C

Final medium: 7 days at 2-8°C in the dark

**Incubation:** Incubate plates at 37+1°C for 24 hours.

**Interpretation:** *Cronobacter sakazakii* appear as green or pale green with a green 'bullseye' centre and 1-2.5mm in size. Other organisms generally appear black (if hydrogen sulphide producers) or colourless.

**References**


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Iversen C, Mullane N, Barbara McCardell, *et al* (2008). "Cronobacter gen. nov., a new genus to accommodate the biogroups of Enterobacter sakazakii, and proposal of Cronobacter sakazakii gen. nov. comb. nov., C. malonaticus sp. nov., C. turicensis sp. nov., C. muytjensii sp. nov., C. dublinensis sp. nov., Cronobacter genomospecies 1, and of three subspecies, C. dublinensis sp. nov. subsp. dublinensis subsp. nov., C. dublinensis sp. nov. subsp. lausannensis subsp. nov., and C. dublinensis sp. nov. subsp. lactaridi subsp. nov.". *IJSEM*.

Lai KK (2001). "*Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature". *Medicine (Baltimore)* **80** (2): 113–22.

# 3. Harmonised Pharmacopoeia (USP/EP/JP)

## Buffered Sodium Chloride-Peptone Solution pH 7.0 (USP/EP/JP)

**HP017**

**Description**

A diluent recommended by the Harmonised European Pharmacopoeia for the microbiological examination of non-sterile pharmaceutical products. The medium is also used to prepare and dilute test suspensions of the microbiological strains described in the Harmonised European Pharmacopoeia. Conforms to USP/EP/JP performance specification. The low level of peptone lessens the physiological shock experienced by micro-organisms when suspended in a diluent. The dual phosphate components create a buffered environment and sodium chloride maintains the osmotic balance.

| Typical Formula | g/litre |
|---|---|
| Potasium dihydrogen phosphate | 3.6 |
| Disodium hydrogen phosphate dihydrate | 7.2 |
| Sodium chloride | 4.3 |
| Peptone (meat or casein) | 1.0 |

**Method for reconstitution**

Disperse 16.1 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Distribute into suitable vessels and sterilise at 121°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, white/buff

Finished medium: colourless, clear to slight haze

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* ATCC 6538
*Bacillus subtilis* ATCC 6633
*Pseudomonas aeruginosa* ATCC 9027
*Clostridium sporogenes* ATCC 19404
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Use:** Test suspensions are made by inoculating the diluent with cultivated micro-organisms. These suspensions must be used within 2 hours if held at room temperature or 24 hours if stored at 2-8°C. No marked increase or decrease in original colony forming unit count should be observed. Refer to the appropriate test protocol for further guidance.

**Interpretation:**

Refer to specific guidelines as defined in the Harmonised European Pharmacopoeia.

**References**

European Pharmacopoeia 8th Edition

## Casein Soya Bean Digest Agar (USP/EP/JP)

**HP016**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the cultivation of a wide range of micro-organisms. Conforms to USP/EP/JP performance specification. The medium is also commonly referred to as tryptone (or tryptic) soy agar and abbreviated to TSA. Enzymatic digests of casein and soya bean act as a source of nitrogen and amino acid and sodium chloride maintains the osmotic balance.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of casein | 15.0 |
| Papaic digest of soya bean | 5.0 |
| Sodium chloride | 5.0 |
| Agar | 15.0 |

**Method for reconstitution**

Disperse 40 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw gel, clear to slight haze

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* ATCC 6538
*Bacillus subtilis* ATCC 6633
*Pseudomonas aeruginosa* ATCC 9027
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Use:** The media is used for the preparation and maintenance of test strains used in the growth promotion test, suitability of the counting methods and negative controls as described in the Harmonised European Pharmacopoeia. It is also a supporting plating medium for various protocols described in the microbial enumeration test section of the Harmonised European Pharmacopoeia.

**Interpretation:**

Cultural response is specific to the test micro-organism. Refer to specific guidelines as defined in the Harmonised European Pharmacopoeia.

**References**

European Pharmacopoeia 8th Edition

## Casein Soya Bean Digest Broth (USP/EP/JP)

**HP002**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the cultivation of a wide range of micro-organisms. Conforms to USP/EP/JP performance specification. The medium is also commonly referred to as tryptone (or tryptic) soy broth and abbreviated to TSB. Enzymatic digests of casein and soya bean act as a source of nitrogen and glucose is a carbon source in the form of a fermentable carbohydrate. Sodium chloride maintains the osmotic balance and dipotassium hydrogen phosphate acts as a buffering agent.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of casein | 17.0 |
| Pancreatic digest of soya bean | 3.0 |
| Sodium chloride | 5.0 |
| Dipotasium hydrogen phosphate | 2.5 |
| Glucose monohydrate | 2.5 |

**Method for reconstitution**

Disperse 30 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Distribute into suitable vessels and sterilise at 121°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: Straw, clear to slight haze

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* ATCC 6538
*Bacillus subtilis* ATCC 6633
*Pseudomonas aeruginosa* ATCC 9027
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Use:**

According to the sterility protocol defined in the Harmonised European Pharmacopoeia the samples are incubated in portions of the medium at 20-25°C for 14 days. No growth of micro-organisms is required for a sterility pass.

According to the growth promotion test defined in the Harmonised European Pharmacopoeia *Bacillus subtilis, Candida albicans* and *Aspergillus brasiliensis* are inoculated (with not more than 100 CFU) and incubated for not more than 3 days in the case of bacteria, and not more than 5 days in the case of fungi. The media is suitable if a clearly visible growth of the micro-organisms occurs.

The media is also used for the preparation of the test micro-organisms *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*. Refer to the specific strain and preparation for incubation time and temperature.

The media is also used for sample preparation and pre-incubation for the test for specified micro-organisms. Refer to individual guidelines for each testing protocol.

**Interpretation:**

Growth is indicated by turbidity. Refer to specific guidelines as defined in the Harmonised European Pharmacopoeia.

**References**

European Pharmacopoeia 8th Edition

## *Cetrimide Agar (USP/EP/JP)*

**HP010**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the isolation and identification of *Pseudomonas aeruginosa*, in non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. Gelatin is a source of nitrogen whilst glycerol acts as a carbon source. Cetrimide is a quarternary ammonium compound that inhibits the growth of a wide range of Gram-positive and some Gram-negative micro-organisms. Magnesium chloride and dipotassium sulphate improve the production of pyoverdin and pyocyanin pigments that combine to give *Pseudomonas aeruginosa* characteristic green colonies. According to the Harmonised European Pharmacopoeia, subculture is carried out onto the medium after enrichment in Casein Soya Bean Digest Broth.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of gelatin | 20.0 |
| Magnesium chloride | 1.4 |
| Dipotassium sulphate | 10.0 |
| Cetrimide | 0.3 |
| Agar | 13.6 |

**Method for reconstitution**

Disperse 45.3 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, add 10mL of glycerol, swirl to mix and boil to dissolve. Sterilise by autoclaving atg121ºC for 15 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: translucent, pale straw gel

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Pseudomonas aeruginosa* ATCC 9027
*Escherichia coli* ATCC 8739

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the European Pharmacopoeia 8.0 subculture is performed from enrichment in casein soya bean digest broth onto the agar surface.

**Incubation:**

Incubate at 30-35°C for 18-72 hours.

**Growth Characteristics**

| Organism | Shape & surface | Colour | Other |
|---|---|---|---|
| *P. aeruginosa* | CV.E.G. | Yellow/green | Fluorescent under UV light |

**References**

European Pharmacopoeia 8th Edition

## *Columbia Agar (USP/EP/JP)*

**HP012**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for isolation and identification of *Clostridia* from non-sterile pharmaceutical products. Conforms to USP/EP/JP performance specification. Originally described as a general purpose nutritious agar base by Ellner et al. at Columbia University that can be enriched by the addition of sterile blood. The peptone mixture and yeast extract provides a source of nitrogen, essential vitamins and amino acids. The starch provides a carbon source and sodium chloride maintains osmotic balance. The Harmonised European Pharmacopoeia states that where necessary, gentamicin sulfate at a concentration of 20mg/L can be added after sterilisation to reduce the growth of non-target organisms. According to the Harmonised European Pharmacopoeia, Reinforced Medium for Clostridia is used as a selective enrichment broth, with subculture performed onto Columbia Agar.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of casein | 10.0 |
| Meat peptic digest | 5.0 |
| Heart pancreatic digest | 3.0 |
| Yeast extract | 5.0 |
| Maize starch | 1.0 |
| Sodium chloride | 5.0 |
| Agar | 15.0 |

**Method for reconstitution**

Disperse 44 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121ºC for 15 minutes. Cool to 45-50°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw clear gel

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms:
*Clostridium sporogenes* ATCC 19404

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the European Pharmacopoeia 8.0 subculture is performed from enrichment in Reinforced Medium for Clostridia onto the agar surface.

**Incubation:**

Incubate at 30-35°C for 48-72 hours.

**Growth Characteristics**

| Organism | Shape & surface | Colour | Other |
|---|---|---|---|
| *C. sporogenes* | CV.E.G. | Buff/cream | The occurrences of anaerobic rods (with or without endospores) giving a negative catalase reaction indicates the presence of *Clostridia* |

**References**

Ellner, P.D., Stoessel, C.J., Drakeford, E. and Vasi, F. (1966). A new culture medium for medical bacteriology. *Amer J. Clin Pathol*. 45. 502-504.

European Pharmacopoeia 8th Edition

## *Enterobacteria Enrichment Broth - Mossel (USP/EP/JP)*

**HP003**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the selective enrichment of bile-tolerant Gram-negative bacteria from non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. Gelatin peptone provides nitrogen, while glucose is a source of fermentable carbohydrate, facilitating efficient growth of most Enterobacteria, including non-lactose fermenting organisms, such as *Salmonella* species. Potassium dihydrogen phosphate and disodium hydrogen phosphate act as a buffer system to encourage early growth and prevent auto sterilisation. Brilliant green and ox bile are selective agents, inhibiting non-target competitive organisms. According to the Harmonised European Pharmacopoeia, Enterobacteria Enrichment (EE) Broth - Mossel is used as a selective enrichment broth, with subculture performed onto Violet Red Bile Glucose Agar (VRBGA).

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of casein | 10.0 |
| Glucose monohydrate | 5.0 |
| Dehydrate ox bile | 20.0 |
| Potassium dihydrogen phosphate | 2.0 |
| Disodium hydrogen phosphate dehydrate | 8.0 |
| Brilliant green | 5.0 |

**Method for reconstitution**

Disperse 45 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and distribute into bottles or tubes. Heat at 100$^{O}$C for 30 minutes in a boiling water bath or flowing steam and cool immediately. DO NOT AUTOCLAVE.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff to light green

Finished medium: green, clear to slight haze

**pH:** 7.2 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* ATCC 8739
*Pseudomonas aeruginosa* ATCC 9027
*Staphylococcus aureus* ATCC 6538

**Hazard classification:** Xi - Irritant

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the Harmonised European Pharmacopoeia, a volume corresponding to 1g of the sample prepared in casein soya bean digest broth pre enrichment is transferred to Enterobacteria Enrichment Broth-Mossel.

**Incubation:**

Incubate at 30-35°C for 24-48 hours.

**Growth Characteristics**

| Organism | Expected result in EE Broth | Expected result on VRBGA subculture |
|---|---|---|
| *E. coli* | Turbid growth | Growth, typical colonies |
| *P. aeruginosa* | Turbid growth | Growth, typical colonies |
| *S. aureus* | No visible growth | No growth |

**References**

European Pharmacopoeia 8th Edition

## *Fluid Thioglycollate Medium (USP/EP/JP)*

**HP001**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for sterility testing. Conforms to USP/EP/JP performance specification. Casein and yeast extract provide a source of nitrogen, essential vitamins and amino acids. The glucose provides a carbon source and sodium chloride maintains osmotic balance. L-Cystine and sodium thioglycollate act as reducing agents to create an anaerobic environment and maintain a low Eh. This is aided by the low level of agar which reduces the oxygen permeability through the medium. Resazurin is an oxidation indicator which turns from colourless to red/pink when oxidised. Sodium thioglycollate also serves to inactivate mercurial compounds. If after sterilisation more than the upper one third of the medium has become red/pink it may be restored once by heating in a water bath or in free-flowing steam until the colour disappears. Ensure the media is cooled quickly and prevent the introduction of non-sterile air into the containers.

| Typical Formula | g/litre |
|---|---|
| L-Cystine | 0.5 |
| Agar | 0.75 |
| Sodium chloride | 2.5 |
| Glucose anhydrous | 5.0 |
| Yeast extract | 2.5 |
| Pancreatic digest of casein | 15.0 |
| Sodium thioglycollate | 0.3 |
| Resazurin | 0.001 |

**Method for reconstitution**

Disperse 26.55 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and bring to the boil. Distribute into suitable vessels and sterilise at 121°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw, clear to slight haze with red/pink layer at the top of the medium

**pH:** 7.1 ± 0.2

Minimum Q.C. organisms:
*Staphylococcus aureus* ATCC 6538
*Bacillus subtilis* ATCC 6633
*Pseudomonas aeruginosa* ATCC 9027
*Candida albicans* ATCC 10231
*Clostridium sporogenes* ATCC 19404
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Use:** According to the sterility protocol defined in the Harmonised European Pharmacopoeia the samples are incubated in portions of the medium at 30-35°C for 14 days. No growth of micro-organisms is required for a sterility pass.

According to the growth promotion test defined in the Harmonised European Pharmacopeia, *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus* are inoculated (with not more than 100 CFU) and incubated for not more than 3 days. The media is suitable if a clearly visible growth of the micro-organisms occurs.

**Interpretation:**

Growth is indicated by turbidity, refer to specific guidelines as defined in the Harmonised European Pharmacopoeia.

**References**

European Pharmacopoeia 8th Edition

## *MacConkey Agar (USP/EP/JP)*

**HP006**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for isolation and identification of *Escherichia coli* from non-sterile pharmaceutical products. Conforms to USP/EP/JP performance specification. Gelatin serves as source of carbon and nitrogen. Lactose is a fermentable carbohydrate and sodium chloride maintains the osmotic balance. Bile salts and crystal violet act as selective agents inhibiting many Gram-positive bacteria. *Escherichia coli* can ferment lactose to produce acid which results in a pH drop. This is indicated by neutral red resulting in pink colonies. Enough acid production will cause the precipitation of bile salts resulting in a bile precipitate or halo around lactose fermenting bacteria. Non-lactose fermenting bacteria such as *Salmonella spp*. grow but remain colourless with no bile precipitate. According to the Harmonised European Pharmacopoeia, MacConkey Broth is used as a selective enrichment broth, with subculture performed onto MacConkey Agar.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of gelatin | 17.0 |
| Peptones (meat and casein) | 3.0 |
| Lactose monohydrate | 10.0 |
| Sodium chloride | 5.0 |
| Bile salts | 1.5 |
| Agar | 13.5 |
| Neutral red | 0.03 |
| Crystal violet | 0.001 |

**Method for reconstitution**

Disperse 50 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Heat to boiling. Sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff to slight red/purple

Finished medium: red/purple, clear gel

**pH:** 7.1 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* ATCC 8739

**Hazard classification:** NR – Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the European Pharmacopoeia 8.0 subculture is performed from enrichment in MacConkey Broth onto the agar surface.

**Incubation:**

Incubate at 30-35°C for 18-72 hours.

**Growth Characteristics**

| Organism | Colour | Other |
|---|---|---|
| Lactose fermenting bile tolerant bacteria | Pink | Red precipitation |
| Non-lactose fermenting bile tolerant bacteria | Colourless | No precipitate |

**References**

European Pharmacopoeia 8th Edition

## *MacConkey Broth (USP/EP/JP)*

**HP005**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the selective enrichment of *Escherichia coli* from non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. Gelatin peptone provides a source of nitrogen, while lactose is a fermentable carbohydrate. Ox bile acts a selective agent inhibiting most Gram-positive organisms and bromocresol purple acts as a pH indicator. A colour change from purple to yellow indicates growth of a bile-tolerant, lactose-fermenting organism such as *Escherichia coli*. According to the Harmonised European Pharmacopoeia, MacConkey Broth is used as a selective enrichment broth, with subculture performed onto MacConkey Agar.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of gelatin | 20.0 |
| Lactose monohydrate | 10.0 |
| Dehydrate ox bile | 5.0 |
| Bromocresol purple | 0.01 |

**Method for reconstitution**

Weigh 35 grams of powder, and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix. Distribute into bottles or tubes, and sterilise at 121°C for 30 minutes

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff to slight yellow

Finished medium: purple, clear to slight haze

**pH:** 7.3 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* ATCC 8739
*Staphylococcus aureus* ATCC 6538

**Hazard classification:** Xi - Irritant

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the Harmonised European Pharmacopoeia, 1mL of casein soya bean digest broth pre enrichment is transferred to 100mL of MacConkey broth.

**Incubation:**

Incubate at 42-44°C for 24-48 hours

**Growth Characteristics**

| Organism | Acid | Gas |
|---|---|---|
| Bile-tolerant lactose fermenters (eg *Escherichia coli*) | Positive | Positive |
| Bile-tolerant non-lactose fermenters (eg *Salmonella typhimurium*) | Negative | Negative |

**References**

European Pharmacopoeia 8th Edition

## *Mannitol Salt Agar (USP/EP/JP)*

**HP009**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for isolation and identification of *Staphylococcus aureus* from non-sterile pharmaceutical products. Conforms to USP/EP/JP performance specification. The peptone and beef extract provide a source of nitrogen, essential vitamins and amino acids. The mannitol is a fermentable carbohydrate and the high level of sodium chloride provides a selective environment favourable to *Staphylococcus aureus*. Phenol red acts as a pH indicator changing from red to yellow when acid is produced by the fermentation of mannitol. The majority of *S. aureus* ferment mannitol producing yellow colonies, occasional strains of coagulase-negative *Staphylococci* may also ferment mannitol. It is necessary to confirm the identity of presumptive *S. aureus* colonies by other means e.g. coagulase, protein A, DN'ase, thermonuclease or latex agglutination. According to the Harmonised European Pharmacopoeia, subculture is carried out onto the medium after enrichment in Casein Soya Bean Digest Broth.

| Typical Formula | g/litre |
|---|---|
| Pancreatic digest of casein | 5.0 |
| Peptic digest of animal tissue | 5.0 |
| Beef extract | 1.0 |
| D-Mannitol | 10.0 |
| Sodium chloride | 75.0 |
| Agar | 15.0 |
| Phenol red | 0.025 |

**Method for reconstitution**

Disperse 111 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and bring to the boil, then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff to slight red

Finished medium: red, clear gel

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms:
*Escherichia coli* ATCC 8739
*Staphylococcus aureus* ATCC 6538

**Hazard classification:** NR - Not Regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the European Pharmacopoeia 8.0 subculture is performed from enrichment in casein soya bean digest broth onto the agar surface.

**Incubation:**

Incubate at 30-35°C for 18-72 hours

**Growth Characteristics**

| Organism | Shape & surface | Colour | Other |
|---|---|---|---|
| *Staphylococcus aureus* | CV.E.G | Yellow | Yellow/white colonies surrounded by a yellow zone |

**References**

European Pharmacopoeia 8th Edition

## *Potato Dextrose Agar (USP/EP/JP)*

**HP015**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the cultivation of fungi and specifically for the preparation of the *Aspergillus brasiliensis* test strain. Conforms to USP/EP/JP performance specification. The medium is commonly abbreviated to PDA. The extract from potato and dextrose provide a nutritionally rich base that encourages mould sporulation and pigment production.

| Typical Formula | g/litre |
|---|---|
| Potato extract | 4.0* |
| *(equivalent to 200g of Infusion from potatoes) | |
| Dextrose | 20.0 |
| Agar | 15.0 |

**Method for reconstitution**

Disperse 39 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw gel, clear to slight haze

**pH:** 5.6 ± 0.2

Minimum Q.C. organisms:
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR - Not Regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Use:** The media is used for the preparation and maintenance of fungal test strains used in the growth promotion test, suitability of the counting methods and negative controls as described in the Harmonised European Pharmacopoeia.

**Interpretation:**

Cultural response is specific to the test micro-organism, refer to specific guidelines as defined in the Harmonised European pharmacopoeia.

**References**

European Pharmacopoeia 8th Edition

# *Rappaport Vassiliadis Salmonella Enrichment Broth (USP/EP/JP)*

**HP007**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the selective enrichment of *Salmonella* from non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. Malachite green and magnesium chloride act as selective agents, combined with high osmotic pressure and low pH to effectively inhibit non-target competitive organisms. Soya peptone provides a source of nitrogen and potassium phosphate acts as a buffer. Some *Salmonella* (*S. typhi* and *S. choleraesuis*) are known to be sensitive to Malachite green and as such may fail to grow. If these organisms are suspected an alternative selective enrichment broth, (e.g. LAB202 Mueller-Kauffmann Tetrathionate novobiocin Broth - MKTTn) should be used in parallel. This formulation is hygroscopic and will produce a slight exothermic reaction when mixed with water. According to the Harmonised European Pharmacopoeia, Rappaport Vassiliadis Salmonella Enrichment Broth is used as a selective enrichment broth, with subculture performed onto Xylose Lysine Deoxycholate (XLD) agar.

| Typical Formula | g/litre |
|---|---|
| Soya peptone | 4.5 |
| Magnesium chloride anhydrous * | 13.58 |
| *equivalent to 29.0g of Magnesium Chloride Hexahydrate | |
| Sodium chloride | 8.0 |
| Dipotassium phosphate | 0.4 |
| Potassium dihydrogen phosphate | 0.6 |
| Malachite green | 0.036 |

**Method for reconstitution**

Weigh 27.14 grams of powder, and add to 1 litre of deionised water. Swirl to dissolve. Distribute into bottles or tubes, and sterilise at 115°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff to light green

Finished medium: blue, clear to slight haze

**pH:** 5.2 ± 0.2

Minimum Q.C. organisms:
*Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028
*Salmonella enterica* subsp. *enterica* serovar Abony ATCC 6017
*Staphylococcus aureus* ATCC 6538

**Hazard classification:** Xi - Irritant

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the Harmonised European Pharmacopoeia, 0.1mL of Casein Soya Bean Digest Broth pre-enrichment is transferred to 10mL of Rappaport Vassiliadis Salmonella Enrichment Broth.

**Incubation:**

Incubate at 30-35°C for 18-48 hours

**Growth Characteristics**

| Organism | Expected result in RV Broth | Expected result on XLD subculture |
|---|---|---|
| *Salmonella spp.* | Turbid growth | Growth, typical colonies |
| *S. aureus* | No visible growth | No growth |

**References**

European Pharmacopoeia 8th Edition

# *Reinforced Medium for Clostridia (USP/EP/JP)*

**HP011**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the selective enrichment of *Clostridia* from non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. The medium is also commonly referred to as Reinforced Clostridial Medium and abbreviated to RCM. Peptone, beef and yeast extract provide a source of nitrogen, essential vitamins and amino acids. Starch aids the detoxification of harmful metabolites and glucose is a fermentable carbohydrate. Sodium chloride provides osmotic balance and sodium acetate acts as a buffer. L-Cysteine act as reducing agents to create an anaerobic environment and maintain a low Eh. This is aided by the low level of agar which reduces the oxygen permeability through the medium. According to the Harmonised European Pharmacopoeia, Reinforced Medium for Clostridia is used as a selective enrichment broth, with subculture performed onto Columbia Agar.

| Typical Formula | g/litre |
|---|---|
| Beef extract | 10.0 |
| Peptone | 10.0 |
| Yeast extract | 3.0 |
| Soluble starch | 1.0 |
| Glucose monohydrate | 5.0 |
| Cysteine hydrochloride | 0.5 |
| Sodium chloride | 5.0 |
| Sodium acetate | 3.0 |
| Agar | 0.5 |

**Method for reconstitution**

Disperse 38 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and bring to the boil. Distribute into suitable vessels and sterilise at 121°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw, clear to slight haze

**pH:** 6.8 ± 0.2

Minimum Q.C. organisms:
*Clostridium sporogenes* ATCC 19404

**Hazard classification:** NR - Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the Harmonised European Pharmacopoeia, a sample is prepared with and without heat treatment and transferred to Reinforced Medium for Clostridia.

**Incubation:**

Incubate at 30-35°C for 48-72 hours

**Growth Characteristics**

| Organism | Expected result in RCM | Expected result on Columbia Agar subculture |
|---|---|---|
| *Clostridia spp.* | Growth | Growth, typical colonies |

**References**

European Pharmacopoeia 8th Edition

## *Sabouraud Dextrose Agar (USP/EP/JP)*

**HP014**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the isolation and identification of *Candida albicans* from non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. The medium is commonly abbreviated to SDA. The medium is also used for the preparation and maintenance of fungal test strains as described by the Harmonised European Pharmacopoeia. The peptone digests and dextrose provide a nutritious base for luxuriant fungal growth and the acidic pH affords selectivity against bacteria. Due to the high carbohydrate content and low pH this medium is highly sensitive to overheating, which will cause a drop in the gel strength. According to the Harmonised European Pharmacopoeia, Sabouraud Dextrose Broth is used as an enrichment broth, with subculture performed onto Sabouraud Dextrose Agar.

| Typical Formula | g/litre |
|---|---|
| Dextrose | 40.0 |
| Peptic digest of animal tissue | 5.0 |
| Pancreatic digest of casein | 5.0 |
| Agar | 15.0 |

**Method for reconstitution**

Disperse 65 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121ºC for 15 minutes. Cool to 45-50°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw gel, clear to slight haze

**pH:** 5.6 ± 0.2

Minimum Q.C. organisms:
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR - Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the European Pharmacopoeia 8.0 subculture is performed from enrichment in Sabouraud Dextrose Broth onto the agar surface.

**Incubation:**

Incubate at 30-35°C for 24-48 hours

**Growth Characteristics**

| Organism | Shape & surface | Colour | Other |
|---|---|---|---|
| *Candida albicans* | CV.E.D. | White | Characteristic odour |

**References**

European Pharmacopoeia 8th Edition

## *Sabouraud Dextrose Broth (USP/EP/JP)*

**HP013**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for the enrichment of *Candida albicans* from non-sterile pharmaceutical samples. Conforms to USP/EP/JP performance specification. The medium is also used for the cultivation of fungal test strains as described by the Harmonised European Pharmacopoeia. The peptone digests and dextrose provide a nutritious base for luxuriant fungal growth and the acidic pH affords selectivity against bacteria. Due to the high carbohydrate content and low pH this medium is highly sensitive to overheating. According to the Harmonised European Pharmacopoeia, Sabouraud Dextrose Broth is used as an enrichment broth, with subculture performed onto Sabouraud Dextrose Agar.

| Typical Formula | g/litre |
|---|---|
| Dextrose | 20.0 |
| Peptic digest of animal tissue | 5.0 |
| Pancreatic digest of casein | 5.0 |

**Method for reconstitution**

Disperse 30 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Distribute into suitable vessels and sterilise at 121°C for 15 minutes.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff

Finished medium: straw, clear to slight haze

**pH:** 5.6 ± 0.2

Minimum Q.C. organisms:
*Candida albicans* ATCC 10231
*Aspergillus brasiliensis* ATCC 16404

**Hazard classification:** NR - Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the Harmonised European Pharmacopoeia, a quantity corresponding to 1g or 1mL of the sample is use to inoculate 100mL of Sabouraud Dextrose Broth.

**Incubation:**

Incubate at 30-35°C for 3-5 days

**Growth Characteristics**

| Organism | Expected result in SDB | Expected result on SDA subculture |
|---|---|---|
| *Candida albicans* | Growth | Growth, white colonies |

**References**

European Pharmacopoeia 8th Edition

## *Violet Red Bile Glucose Agar (USP/EP/JP)*

**HP004**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for isolation and identification of bile-tolerant Gram-negative bacteria from non-sterile pharmaceutical products. Conforms to USP/EP/JP performance specification. Yeast extract provides vitamins and gelatin serves as source of carbon and nitrogen. Glucose is a fermentable carbohydrate and sodium chloride maintains the osmotic balance. Bile salts and crystal violet act as selective agents inhibiting many Gram-positive bacteria. The formulation is a modification of Violet Red Bile Agar by Mossel which substitutes lactose for glucose. *Enterobacteriaceae,* such as *Escherichia coli* and *Salmonella spp*., are able to ferment glucose. This produces acid which results in a pH drop indicated by neutral red resulting in pink colonies. Enough acid production will cause the precipitation of bile salts resulting in a bile precipitate or halo around glucose fermenting bacteria. Non-glucose fermenting bile tolerant bacteria such as *Psuedomonas aeruginosa* grow but remain colourless with no bile precipitate. According to the Harmonised European Pharmacopoeia, Enterobacteria Enrichment Broth-Mossel is used as a selective enrichment broth, with subculture performed onto Violet Red Bile Glucose Agar (VRBGA).

| Typical Formula | g/litre |
|---|---|
| Yeast extract | 3.0 |
| Pancreatic digest of gelatin | 7.0 |
| Bile salts | 1.5 |
| Sodium chloride | 5.0 |
| Glucose monohydrate | 10.0 |
| Agar | 15.0 |
| Neutral red | 0.03 |
| Crystal violet | 0.002 |

**Method for reconstitution**

Disperse 41.5 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Heat to boiling. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff to slight red/purple

Finished medium: red/purple, clear gel

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms:
*Pseudomonas aeruginosa* ATCC 9027
*Escherichia coli* ATCC 8739

**Hazard classification:** NR - Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the European Pharmacopoeia 8.0 subculture is performed from enrichment in Enterobacteria Enrichment Broth-Mossel onto the agar surface.

**Incubation:**

Incubate at 30-35°C for 18-24 hours

**Growth Characteristics**

| Organism | Colour | Other |
|---|---|---|
| Glucose-fermenting bile-tolerant Gram-negative bacteria | Pink | Red precipitate |
| Non glucose-fermenting bile-tolerant Gram-negative bacteria | Colourless | No precipitate |

**References**

Mossel, D.A.A. Media for Enterobacteriaceae (1985) International Journal of Food Microbiology, 2 (1-2), pp. 27-32.

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## *Xylose Lysine Deoxycholate Agar (USP/EP/JP)*

**HP008**

**Description**

A medium recommended by the Harmonised European Pharmacopoeia for isolation and identification of *Salmonella* from non-sterile pharmaceutical products. Conforms to USP/EP/JP performance specification. Originally formulated by Taylor to differentiate enteric pathogens, the agar is widely used as the preferred differential medium for *Salmonella spp*. The medium is void of peptones but instead uses yeast extract as a carbon, nitrogen and vitamin source and xylose, lactose and sucrose are fermentable carbohydrates. *Salmonella* are able to ferment xylose to produce acid but not lactose or sucrose. When the xylose is exhausted *Salmonella* will decarboxylate lysine shifting the pH back to neutral. At near neutral pH, *Salmonella* can reduce sodium thiosulfate producing hydrogen sulfide which creates a complex with ferric ammonium citrate to produce black or black centred colonies. Other organisms are able decarboxylate lysine but acid production from the fermentation of lactose and sucrose keeps the pH too acidic for $H_2S$ production. Selectivity is achieved through the incorporation of sodium deoxycholate and phenol red acts as a pH indicator. According to the Harmonised European Pharmacopoeia, Rappaport Vassiliadis Salmonella Enrichment Broth is used as a selective enrichment broth, with subculture performed onto Xylose Lysine Deoxycholate (XLD) agar.

| Typical Formula | g/litre |
|---|---|
| Xylose | 3.5 |
| L-Lysine | 5.0 |
| Lactose monohydrate | 7.5 |
| Sucrose | 7.5 |
| Sodium chloride | 5.0 |
| Yeast extract | 3.0 |
| Phenol red | 0.08 |
| Agar | 13.5 |
| Sodium deoxycholate | 2.5 |
| Sodium thiosulfate | 6.8 |
| Ferric ammonium citrate | 0.8 |

**Method for reconstitution**

Disperse 55 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes then swirl to mix. Heat to boiling. Cool to 50°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

**Appearance:**

Powder: fine, free-flowing, homogeneous, buff to slight red

Finished medium: red, clear gel

**pH:** 7.4 ± 0.2

Minimum Q.C. organisms:
*Salmonella typhimurium* ATCC 14028
*Salmonella abony* ATCC 6017

**Hazard classification:** NR - Not regulated

**Storage:**

Dehydrated culture media: 10-25°C away from direct sunlight.

Prepared media: 7 days at 2-8°C in the dark.

**Inoculation:** According to the European Pharmacopoeia 8.0 subculture is performed from enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth onto the agar surface

**Incubation:**

Incubate at 30-35°C for 18-48hours

**Growth Characteristics**

| Organism | Shape & surface | Colour | Other |
|---|---|---|---|
| *Salmonella spp.* | CV.E.G. | Red | Black or black centered colonies |

**References**

Taylor, W. I. 1965. Isolation of shigellae. I. Xylose lysine agars: new media for isolation of enteric pathogens. Am. J. Clin. Pathol. 44 (4):471-475.

European Pharmacopoeia 8th Edition

# 4. Biomolecular Products

| | |
|---|---|
| **LB Agar** | **YPD Agar** |
| **LB Broth** | **YPD Broth** |
| **Harlequin™ LB agar*** | **2 x YT Agar** |
| **Harlequin™ LB Top*** | **2 x YT Broth** |
| **LB Agar (Lennox)** | **NZY Broth** |
| **LB Broth (Lennox)** | **NZCYM Broth** |
| **Terrific Broth** | **Luria Bertani Agar (Hi-Salt)** |

*Available from Lab M in the UK only. Outside the UK Harlequin LB agar is available from Sigma-Aldrich as S-Gal LB agar (C4478).

**Lab M's** Biomolecular products form the basis of gene reporter assays that employ enzyme substrates, such as X-gal and our patented CHE-gal, to indicate inactivation of α-complementation. These products are formulated to promote the growth of the recipient and donor cells used in DNA insertion technology. More importantly, they are formulated to provide optimum conditions for plasmid retention or bacteriophage reproduction and survival.

As different applications have varied requirements of the culture medium used, Lab M offer a range of media types. Some are of standard formulation whilst others are modified to enhance the performance of specific applications. This variety allows the researcher to choose the appropriate medium for the application being used.

Lab M have formulated unique versions of LB Agar and LB Top Agar which incorporate the patented water soluble chromogen CHE-β-gal into the complete medium. This improves colour definition of α-complemented colonies compared to the standard X-β-gal plate and removes the need for hazardous chemicals in the preparation of the medium. Therefore we have produced a safe, fast and easy way to differentiate between $lac^+$and $lac^-$ colonies. Simply add water to the powder and autoclave. All products are available directly from Lab M in the UK.

## *Harlequin™ LB Agar*

**HAL004**

**Description**
A nutritious molecular biology medium containing the novel chromogen CHE-galactoside to enable rapid, safe and unambiguous detection of plasmid transformed bacteria. The CHE-galactoside replaces the traditional X-gal substrate, simplifying the technique as there is no preparation of stock solutions in dimethyl formamide or dimethyl sulphoxide and surface application of the chromogen to the medium. The intense black colour of the colonies gives a sharper contrast between $lac^+$and $lac^-$colonies, giving improved colony detection compared to blue X-gal stained colonies.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 10.0 |
| CHE-galactoside | 0.3 |
| IPTG | 0.03 |
| Ferric ammonium citrate | 0.5 |
| Agar | 12.0 |

**Method for reconstitution**
Weigh 37.8 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add appropriate filter sterilised antibiotic if required. Pour into sterile Petri dishes, allow the medium to set and dry the surface prior to inoculation.

**Appearance:** Straw, clear gel.

**pH:** 7.0 ± 0.2

Minimum QC organisms - ß-gal reaction
*Escherichia coli* DH5a (ATCC®53868) Lac Z+ve (black)
*Escherichia coli* DH5a, Lac Z -ve (remains cream)

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C in the dark.

**Inoculation:** Typically surface spread over plate to detect cream colonies indicating disruption of β-complementation. Alternatively, spread for single colonies if required.

**Incubation:** 37°C aerobically, for 16-18 hours. The colour of the colonies will substantially increase with prolonged incubation (up to 24 hours).

**Interpretation:** Examine for the presence of cream colonies, which indicates a successful insertion of the target DNA.

**References**

Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New ,York.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

## *LB Agar*

**LAB168**

**Description**
A nutritious medium designed for rapid bacterial growth, typically used in molecular biology procedures e.g. in the detection of phage or plasmid transformed bacteria and the maintenance of recombinant strains. This agar contains the required concentration of sodium chloride to promote replication of plasmids.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 10.0 |
| Agar | 15.0 |

**Method for reconstitution**
Weigh 40.0 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add filter sterilised antibiotic if required. Pour into sterile Petri dishes and allow the medium to set. Dry the surface prior to inoculation.

**Addition of Substrate**
Prepare the X-Gal solution by dissolving in DMF, to give a concentration of 20mg/ml Once dissolved, spread 40µl as a surface layer over the top of the agar and allow to dry. Also spread 4µl of a solution of IPTG (200mg/ml). Alternatively, use Harlequin™ LB agar complete (HAL004), which already contains the enzyme substrate and inducer. This eliminates the potentially hazardous use of DMF and prevents variation in the colour of ß-complemented colonies due to differences in substrate concentration.

**Appearance:** Straw, clear gel.

**pH:** 7.0 ± 0.2

Minimum QC organisms - ß-gal reaction:
*Escherichia coli* DH5a (ATCC 53868)
*Lac Z+ve* (black if CHE-gal is present in the medium)
*Escherichia coli* DH5a, Lac Z -ve (remains cream even in the presence of CHE-gal)

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C in the dark.

**Inoculation:** Typically surface spread over plate to detect cream colonies indicating disruption of ß-complementation. Alternatively, spread for single colonies if required.

**Incubation:** 37°C aerobically, for 16-18 hours. If a chromogenic substrate is used, the colour of the colonies will substantially increase with prolonged incubation (up to 24 hours).

**Interpretation:** Using the base medium alone, all colonies will appear cream. Alternatively, if a chromogen is included, examine for the presence of cream colonies, which indicates a successful insertion of the target DNA.

**References**

Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New ,York.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

## *LB Agar (Lennox)*

**LAB174**

**Description**

This is a nutritionally rich medium containing half the sodium chloride level of LB agar (LAB168). This allows the researcher to select the optimum salt concentration for his experiment. This medium can also be used for plasmid replication experiments. Nutritionally rich media are required for molecular biology applications as the strains used are often derived from *Escherichia coli* K12, which is deficient in B vitamin production.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 5.0 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 35.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add filter sterilised antibiotic as required. Pour into sterile Petri dishes and allow the medium to set. Dry the surface prior to inoculation.

**Addition of Substrate**

Prepare the X-Gal solution by dissolving in DMF, to give a concentration of 20mg/ml. Once dissolved, spread 40µl as a surface layer over the top of the agar and allow to dry. Also spread 4µl of a solution of IPTG (200mg/ml).

**Appearance:** Straw, clear gel.

**pH:** 7.0 ± 0.2

Minimum QC organisms - ß-gal reaction:
*Escherichia coli* DH5a (ATCC 53868)
*Lac Z+ve* (black if CHE-gal is present in the medium)
*Escherichia coli* DH5a, Lac Z -ve (remains cream even in the present of CHE-gal)

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C in the dark.

**Inoculation:** Surface; either spread over entire surface for colony count or streaking for single colonies.

**Incubation:** 37°C aerobically for 16-18 hours.

**Interpretation:** Using the base medium alone, all colonies will appear cream. Alternatively, if a chromogen is included, examine for the presence of cream colonies, which indicates a successful insertion of the target DNA.

**References**

Lennox, E.S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1, 190.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman. J.A., Smith, J.G. and Struhl. (1994). Current protocols in molecular biology. Vol. 1. Current protocols, New York. N.Y.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

## *LB Broth*

**LAB169**

**Description**

A nutrient broth primarily used for the growth and maintenance of *Escherichia coli*. Used as the primary propagation step for donor or recipient cells, when further work is to be performed on LB Agar. This broth contains a high level of sodium chloride to aid the maintenance of plasmids. If working with temperate bacteriophages, such as lambda, the addition of magnesium sulphate ($MgSO_4.7H_2O$) at 2 grams per litre is recommended to promote phage absorption.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 10.0 |

**Method for reconstitution**

Weigh 25.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Straw, clear liquid.

**pH:** 7.0 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** As per normal techniques, using a pure culture of donor/recipient cells.

**Incubation:** 37°C aerobically for 16-18 hours.

**Interpretation:** Examine all tubes for turbidity, indicating growth.

**References**

Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New ,York.

Sambrook, J., Fritsch, E.F. and Maniatis. T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

## *LB Broth (Lennox)*

**LAB173**

**Description**

This is a nutrient broth containing half the sodium chloride level of LB Broth (LAB169), this allows for the addition of calcium chloride, required in some applications for efficient phage adsorption to the cell e.g. phage P1. This medium can also be used for plasmid replication experiments. Chloramphenicol can be added to achieve high plasmid copy number by inhibiting chromosomal replication.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 5.0 |

**Method for reconstitution**

Weigh 20.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Straw, clear liquid.

**pH:** 7.5 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** As per normal techniques, using a pure culture of donor/recipient cells.

**Incubation:** 37°C aerobically for 16-18 hours.

**Interpretation:** Examine all tubes for turbidity, indicating growth.

**References**

Lennox, E.S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1, 190.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman. J.A., Smith, J.G. and Struhl. (1994). Current protocols in molecular biology. Vol. 1. Current protocols, New York. N.Y.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

## *Luria Bertani (Hi-Salt) Broth*

**LAB191**

**Description**

A nutritious medium designed for rapid bacterial growth, typically used in the detection of phage or plasmid transformed bacteria. This broth is formulated to LB Broth (LAB169), but has a higher pH for different applications.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 10.0 |
| Yeast Extract | 5.0 |
| Sodium chloride | 10.0 |

**Method for reconstitution**

Weigh 25.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Straw, clear gel.

**pH:** 7.5 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Dependent upon application.

**Incubation:** 37°C aerobically.

## *NZCYM Broth*

**LAB182**

**Description**

This is an improved medium for increased yields of the phage Lambda. This formulation includes a higher concentration of essential elements for increased bacterial growth. To encourage multi phage insertion into the host cell, it is recommended to add 0.2% maltose (prepare a 20% solution and add 1ml per 100ml of medium), which promotes expression of *LamB* (lambda receptor). If maltose is added, do not use this medium to create phage stocks, as binding of phage particles to membrane fragments will occur because of increased *LamB* density.

| Typical Formula | g/litre |
|---|---|
| Enzymatic casein digest | 10.0 |
| Acid hydrolysed casein | 1.0 |
| Yeast extract | 5.0 |
| Magnesium Sulphate | 2.0 |
| Sodium chloride | 5.0 |

**Method for reconstitution**

Weigh 23.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Straw, clear liquid.

**pH:** 7.0 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Mix a fresh overnight culture of host cells with bacteriophage and use to inoculate NZCYM Broth.

**Incubation:** 37°C aerobically.

**References**

Blattner, F., *et al.*, (1977) Science 196, 161.

## *NZY Broth (NZYM)*

**LAB181**

**Description**

Designed for increased replication of phage Lambda. To encourage multi phage insertion into the host cell, it is recommended that 0.2% maltose be added (prepare a 20% solution and add 1ml per 100ml of medium), which promotes expression of *LamB* (lambda receptor). If maltose is added, the medium should not be used to create phage stocks, as binding of phage particles to membrane fragments will occur because of increased *LamB* density.

| Typical Formula | g/litre |
|---|---|
| Enzymatic casein digest | 10.0 |
| Yeast extract | 5.0 |
| Magnesium Sulphate | 2.0 |
| Sodium chloride | 5.0 |

**Method for reconstitution**

Weigh 22.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Straw, clear liquid.

**pH:** 7.0 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Mix a fresh overnight culture of host cells with bacteriophage and use to inoculate NZY Broth.

**Incubation:** 37°C aerobically for 16-18 hours.

**References**

Blattner, F., *et al.*, (1977) Science 196, 161.

## *Terrific Broth*

**LAB183**

**Description**

A nutritious medium that will support high bacterial cell densities, usually resulting in increased yields of DNA and recombinant proteins. The formulation requires the addition of glycerol to complete the formulation.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 12.0 |
| Yeast extract | 24.0 |
| Di Potassium phosphate | 9.4 |
| Potassium di phosphate | 2.2 |
| Glycerol (added after autoclaving). | 4.0/8.0 ml |

**Method for reconstitution**

Weigh 47.6 grams of powder and disperse in 1 litre of deionised water. Add 4.0 or 8.0 ml of glycerol, swirl to mix and dispense into final containers. Allow the mixture to soak for 10 minutes, swirl to dissolve and sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Straw, clear liquid.

**pH:** 7.0 ± 0.2

Minimum QC organisms: *Escherichia coli* DH5 (ATCC 53868)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Inoculate with a pure culture of the host strain containing the required recombinant plasmid.

**Incubation:** 37°C aerobically.

**References**

Tartoff, C.D. and Hobbs, C.A. (1987). Improved media for growing plasmid and cosmid clones. Bethesda Res. Lab. Focus, 9:205.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.

## *YPD Agar*

**LAB176**

**Description**

A nutritious medium used as an alternative to YPD Broth, where a solid base is required. Glucose is included to promote rapid growth.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Yeast extract | 10.0 |
| Glucose | 20.0 |
| Agar | 17.0 |

**Method for reconstitution**

Weigh 67.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add filter sterilised antibiotic as required. Pour into sterile Petri dishes and allow to set. Dry the surface prior to inoculation.

**Appearance:** Dark straw, clear liquid.

**pH:** 6.5 ± 0.2

Minimum QC organisms: *Saccharomyces cerevisiae*

**Inoculation:** Surface; either spread over entire surface for colony count or streaking for single colonies.

**Incubation:** 37°C aerobically for 16-18 hours.

**Interpretation:** Yeasts will grow as cream colonies, size dependent upon inoculum density.

## *YPD Broth*

**LAB175**

**Description**

A nutritious broth base recommended for the maintenance and propagation of yeasts widely used in gene insertion techniques. Glucose is included to promote rapid growth.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Yeast extract | 10.0 |
| Glucose | 20.0 |

**Method for reconstitution**

Weigh 50.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and dispense into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Dark straw, clear liquid.

**pH:** 6.5 ± 0.2

Minimum QC organisms: *Saccharomyces cerevisiae*

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** As per normal techniques, using a pure culture of strain to be cultivated.

**Incubation:** 37°C aerobically for 16-18 hours.

**Interpretation:** Examine all tubes for turbidity, indicating growth.

## *2xYT Agar*

**LAB180**

**Description**

An agar version of 2xYT broth, for the growth of host cells of filamentous single stranded bacteriophages e.g. the M13 phage.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 16.0 |
| Yeast extract | 10.0 |
| Sodium chloride | 5.0 |
| Agar | 15.0 |

**Method for reconstitution**

Weigh 46.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C, pour into sterile Petri dishes and allow the medium to set. Dry the surface prior to inoculation.

**Appearance:** Straw, clear gel.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *Escherichia coli* DH5 (ATCC 53868).

**Storage of Prepared Medium:** Plates – up to 7 days at 2-8°C in the dark.

**Inoculation:** dependent upon application.

**Incubation:** 37˚C aerobically.

---

## *2xYT Broth*

**LAB179**

**Description**

A nutritious liquid medium formulated to promote the growth of host cells, thereby encouraging increased replication and yield from filamentous single stranded bacteriophages (such as the M13 phage).

| Typical Formula | g/litre |
|---|---|
| Tryptone | 16.0 |
| Yeast extract | 10.0 |
| Sodium chloride | 5.0 |

**Method for reconstitution**

Weigh 31.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes.

**Appearance:** Straw, clear liquid.

**pH:** 7.0 ± 0.2

Minimum Q.C. organisms: *Escherichia coli* DH5 (ATCC 53868).

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** Mix fresh overnight culture of host cells with bacteriophage and use to inoculate 2xYTBroth.

**Incubation:** 37°C aerobically, typically for 4-5 hours to reduce risk of selecting deletion mutants.

**Interpretation:** Concentrate bacterial cells by centrifugation and transfer supernatant containing bacteriophage to a fresh tube, being careful not to disturb the pellet formed. The resulting bacteriophage stock can be stored at +4°C or -20°C.

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# 5. µPREP™ Ready-to-Reconstitute Media

µPREP™ is a line of bagged, sterile dehydrated microbiological culture media that are made ready to use simply by adding water; no autoclaving required.

Using Lab M's proven dehydrated culture media formulations, µPREP™ is designed primarily for high throughput laboratories where speed, convenience, reliability and cost-effectiveness are high priorities, and where storage space is often at a premium. µPREP™ Half Fraser Broth ISO (+FAC) has the added advantage that all supplements, including ferric ammonium citrate normally added manually after autoclaving, are added, meaning Technicians do not need to add any supplements at all.

µPREP™ BPW (ISO) and µPREP™ Half Fraser Broth ISO (+FAC) are supplied sterile in boxes of 10 highly robust bags, each of which makes 20 litres of prepared medium.

µPREP™ bags are connected to a reverse osmosis (RO)/deionised water supply and the water is sterilised as it is pumped via a membrane filter into the bag.

## µPREP™ BPW (ISO)

**MPB001**

**Description**

Formulated to ISO 6579, Buffered Peptone Water (ISO) is a pre-enrichment medium designed to help sublethally damaged salmonellae recover before introducing them into a selective medium. This nutrient medium is free from inhibitors and is well buffered to maintain pH 7.0 for the incubation period. Sublethal injury to salmonellae occurs in many food processes and this pre-enrichment step greatly increases recovery of these organisms.

| Typical Formula | g/litre |
|---|---|
| Enzymatic digest of casein | 10.0 |
| Sodium chloride | 5.0 |
| Disodium hydrogen phosphate (anhydrous) | 3.6* |
| Potassium dihydrogen phosphate | 1.5 |

*Equivalent to 9.0g of disodium hydrogen phosphate dodecahydrate

**Method for reconstitution**

Each µPREP™ BPW (ISO) bag contains sufficient media to prepare 20 litres BPW (ISO). µPREP™ bags should be hydrated by addition of 20 litres RO/deionised water via a membrane filter.

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Salmonella Typhimurium* WDCM 00031
*Staphylococcus aureus* WDCM 00034
*Listeria monocytogenes* WDCM 00021

**Storage**

Bag (as supplied): store in the dark at 10-25ºC

Bag (reconstituted): store in the dark at 10-25ºC for up to 72 hours (providing asepsis is maintained)

**References**

BS EN ISO 6579:2002 Microbiology of food and animal feeding stuffs – Horizontal method for the

detection of Salmonella spp. (Incorporating Corrigendum No. 1)

## µPREP™ Half Fraser Broth ISO (+FAC)

**MPB004**

**Description**

µPrep™ Half Fraser Broth ISO (+FAC) is a sterile, ready to reconstitute primary enrichment medium for the isolation of Listeria spp. from foodstuffs formulated according to ISO 11290.

Listeria spp. are able to hydrolyse the aesculin to form aesculetin, which reacts with the FAC resulting in a black precipitate and a visible positive reaction. However, Enterococci can also perform this reaction, so further plating is required onto an isolation medium such as Harlequin™ Listeria Chromogenic Agar ISO (HAL010) or Pinnacle™ Listeria Chromogenic Agar ISO (PIN001). Selectivity is provided by lithium chloride, acriflavine and nalidixic acid.

| Typical Formula | g/litre |
|---|---|
| Peptone mixture | 15.0 |
| Yeast extract | 5.0 |
| Aesculin | 1.0 |
| Disodium hydrogen phosphate | 9.6 |
| Potassium dihydrogen phosphate | 1.35 |
| Socium chloride | 20.0 |
| Lithium chloride | 3.0 |
| Acriflavin | 0.0125 |
| Nalidixic acid | 0.01 |
| Ferric ammonium citrate | 0.5 |

**Method for reconstitution**

Each µPREP™ Half Fraser Broth ISO (+FAC) bag contains sufficient media to prepare 20 litres complete Half Fraser Broth. µPREP™ bags should be hydrated by addition of 20 litres RO/deionised water via a membrane filter.

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Enterococcus faecalis* WDCM 00087
*Listeria monocytogenes* WDCM 00021

**Storage**

Bag (as supplied): store in the dark at 10-25ºC

Bag (reconstituted): store in the dark at 10-25ºC for up to 72 hours (providing asepsis is maintained)

**References**

Fraser, J.A. and Sperber, W.H. (1988). Rapid detection of Listeria spp in food and environmental samples by esculin hydrolysis. J. Food Protect. 51, No.10, 762-765.

McClain, D. and Lee, W.H. (1989). FSIS method for isolation of L. monocytogenes from processed meat and poultry products. Lab. Comm.No.57, Revised May 24, (1989). US Dept of Agric.FSIS,

Microbiol. Div.

ISO 11290-1:1996+A1:2004, Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of Listeria monocytogenes. Detection method

# µPREP™ Accessories

## µPREP™ Filter Unit

**MPA001**

Used with Lab M µPREP™ bags, the µPREP™ Filter Unit is a device for the filter-sterilisation of reverse osmosis (RO)/deionised water. The µPREP™ Filter Unit should be sterilised prior to use. µPREP™ Filter Units may be sterilised and reused for upto 100 litres or 5 filled (20 litre) bags.

## µPREP™ Quick Connectors

**MPA002**

Used with Lab M µPREP™ bags, the µPREP™ Quick Connectors may be attached to tubing sets for the dispensing of reconstitued media. The µPREP™ Quick Connectors should be attached to tubing and sterilised prior to use. µPREP™ Quick Connectors may be rinsed, resterilised and reused.µPREP™ Filter Units may be sterilised and reused for upto 100 litres or 5 filled (20 litre) bags.

# 6. Captivate™ Immunomagnetic Separation

***Captivate***™ is a range of antibody coated paramagnetic particles for the specific immunomagnetic separation (IMS) of microorganisms.

This patented technology consists of microscopic paramagnetic particles. The beads have a magnetite core and a "ceramic" zirconium oxide coating. The beads are manufactured by a high speed blending process and typically cover a size diameter range of 1-4 µm, with a 2.5µm average size.

Purified antibodies to surface components of the target microorganism are covalently coupled to the bead. With careful antibody selection, a highly specific separation system for microorganisms is produced.

The pre-coated beads are designed for the IMS of target bacteria from enrichment cultures. A sample is taken from a filter stomacher bag and incubated with the ***Captivate***™ beads for 30 minutes. The bead/ microorganism complexes are then removed from the solution by placing the sample tube in a Captivate separator rack (CAP100-12P). This separates them from the background organisms and interfering materials. The complexes are then washed using a PBS/ Polysorbate 20 wash buffer to remove non-specifically bound material. The beads can then be plated out onto the appropriate selective agar media and incubated as described.

The IMS technique will increase the sensitivity and specificity of the methodology and, in most circumstances, results can be achieved 24 hours earlier than standard protocols.

These products can also serve as a capture system for rapid detection systems.

**Special Notes on IMS Techniques.**

There are important factors that affect the performance of IMS techniques. Thorough mixing of the particles and sample allied with efficient recovery of the beads from the sample matrix is paramount to the success of this technique. Care must be taken not to aspirate the sample vigorously as this can result in the loss of captured target organisms. Certain sample types (e.g. very fatty, particulate and viscous samples) can interfere with bead recovery. To counter-act this interference, samples can be diluted in PBS-Polysorbate e.g. 1:2-1:4, reducing the effect of the matrix and allowing more efficient bead recovery. Alternatively with problem samples, after the initial magnetic separation the incomplete removal of the sample (i.e. remove 800µl) and continuation of the wash protocol as described can minimise bead losses.

***Captivate***™ **Product Specification**

| | |
|---|---|
| Working concentration: | Typically 5mg/ml |
| $Fe_3O_4$content: | 29-33% w/w |
| Antibody: | Particles coated with high avidity, affinity purified and absorbed polyclonal antibodies to cell surface antigens. |
| Specificity: | Reacts with target organism. |
| Average size: | 2.5 µm (typical range 1-8µm) |
| Formulation: | Particles are suspended in PBS plus1% BSA pH 7.3-7.5 and 0.09% azide as preservative. |
| Storage: | 8°C (may be shipped at ambient) |
| Shelf life: | 2 years. |

**Generic *Captivate*™ IMS Procedure**

1. Add 20µl of well mixed ***Captivate***™ particles to a suitable micro tube (1.5 - 2.5ml volume).
2. To this add 1ml of the enrichment culture taking care to avoid transfer of sample debris.
3. Cap tube tightly and rotamix the suspension for 30 minutes at room temperature.
4. Insert tube into magnetic separator rack for 3 minutes to concentrate the beads to a pellet. Gently invert the rack several times to aid pelleting of the beads.
5. Carefully aspirate the supernatant from the tube and cap without removing particles, taking care to avoid splashing.
6. Remove magnet from rack or tubes from the rack and add 1ml of wash. Cap and resuspend particles by inverting several times.
7. Repeat separation and wash steps 4-6 twice more. Finally resuspend particles in 100µl of wash.
8. Remove 50µl of the complexed, resuspended particles to the plating media, streaking for single colonies. Incubate plates at 37°C for 18-24 hours and examine for typical colonies.

**Phosphate Buffered Saline plus Polysorbate** .

| Typical Formula | g/litre |
|---|---|
| Sodium chloride | 8.0 |
| Potassium chloride | 0.020 |
| Disodium hydrogen phosphate | 1.15 |
| Potassium dihydrogen phosphate | 0.2 |
| Polysorbate 20. | 0.5 |

**pH:** 7.0 ± 0.2

Dissolve the components in deionised water and check the pH. Sterilise the solution by autoclaving at 121°C for 15 min. Allow the solution to cool and check the pH. Store in the dark and use within one month.

## *Captivate*™ *O157*

**CAP001**

**Description**

***Captivate***™ O157 are magnetisable particles coated with specific antibody intended for the isolation of *E. coli* O157:H7 from food, animal feeds, beverages, pharmaceutical or environmental samples. The particles help to concentrate O157:H7 cells in mixed culture reducing the probability of missing low numbers or overgrowth of O157:H7 colonies by competing flora. In fact, immunomagnetic separation is now regarded as the gold standard method for isolation of *E. coli* O157:H7 from food and environmental samples.

*E. coli* O157:H7 is the primary serovar associated with food borne gastrointestinal infection, resulting in self-limiting diarrhoea, that can lead to serious disease conditions such as haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP).

The organism itself is associated with raw meats and unpasteurised milk[1], probably due to the implication of farm animals and particularly cattle as carriers of *E. coli* O157:H7. Large outbreaks have been recorded in the United States from consumption of unpasteurised apple juice (apple cider) possibly as a result of using apples which have fallen to the ground where the potential for contamination with the organism exists[3,4].

**Enrichment Protocol for *E. coli* O157:H7**.

The recommended protocol for the isolation of *E. coli* O157:H7 employs a 6 hour enrichment step at 42°C in modified Tryptone Soy Broth (mTSB, LAB165) plus novobiocin (X150) followed by IMS (see below) and plating onto Sorbitol MacConkey Agar (LAB161 or HAL006) supplemented with or without the addition of cefixime and potassium tellurite (X161)[5-8]. It is also recommended that a further IMS and inoculation of SMAC plates is performed after incubation of the sample for 24 hours. Alternative enrichment protocols using different media have been described e.g. Buffered Peptone Water (LAB046) plus VCC (X546)[5-8].

Follow the ***Captivate***™ protocol as outlined earlier in points 1 to 8.

**Interpretation:** Examine the SMAC and CTSMAC plates for typical *E. coli* O157 non-sorbitol fermenting colonies that are smooth and circular, 1-3 mm in diameter that are colourless to pale orange. Confirm the colony identity with commercially available latex agglutination kits or antisera.

**Product Presentation**

***Captivate***™ O157 is available in packs of 50 test, product code CAP001-050 and 250 test, product code CAP001-250. Materials required, but not provided, include phosphate buffered saline-Polysorbate 20, pipettes and tips, stomacher machine and bags, magnetic separator rack and culture media. Magnetic separating racks (CAP-100-12P) and rotating mixers (CAP101-58) are also available from LAB M.

**Reference**

1) Padhye, N.V., and Doyle, M.P. (1992). *Escherichia coli* O157:H7: Epidemiology, Pathogenesis and Methods for Detection in Food. J.Food.Prot. **55**, 555-565.

2) Martin, M.L. *et al* (1986) Isolation of *Escherichia coli* from cattle associated with two cases of hemolytic syndrome. Lancet **ii** 1043.

3) Besser, R.E. *et al* (1993) An outbreak of diarrhoea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA **259** 2217-2220

4) McCarthy, M. (1996) *E. coli* O157:H7 outbreak in USA traced to apple juice. Lancet **348** 1299.

5)Wright, D.J., Chapman, P.A. and Siddons, C.A. (1994). Immunomagnetic separation as a sensitive method for the isolation of *Escherichia coli* O157 from food samples. Epidemiology and Infection **113**, 31-39.

6) Bolton, F.J.; Crozier, L.; Williamson, J.K. (1995) New technical approaches to *Escherichia coli* O157. PHLS Microbiol. Dig. **12** 67-71.

7) Vernozy-Rozand, C. (1997). Detection of *Escherichia coli* O157 and other VTEC in food. Journal of Applied Microbiology. **82**, 537-551.

8) Ogden, I.D.; Hepburn, N.F.; & MacRae, M. (2001). The optimisation of media used in the immunomagnetic separation methods for the detection of *Escherichia coli* O157 in foods. J. Appl.Mic. 91, 373-379.

## *Non-O157 Shiga Toxin-Producing Escherichia coli (STEC)*

Since E. coli O157:H7 was first identified, shiga-toxin producing Escherichia coli (STEC, also referred to as VTEC - verocytotoxin-producing E. coli) of various serotypes have become an ncreasing concern for public health. Numerous outbreaks have been attributed to STEC serotypes and symptoms may includes bloody or acute diarrhoea, and/or the development in some patients of Haemolytic Ureamic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP).

Non-O157 VTEC are associated with the following: fresh meat; ready-to-eat fermented meats (e.g. salami, pepperoni); fresh produce (ready-to eat pre-cut vegetables and sprouted seeds); raw and low heat-treated milk and derived dairy products; and in the hides and fleeces of cattle, sheep and goats.

Reference methods, including ISO/TS 13136:2012 and MLG 5B.05, include immunomagnetic capture and separation for the key serogroups. This is because the different serogroups cannot be differentiated on agar without this technique. Subsequently immunomagnetic separation results in improved rates of isolation.

Lab M offer an extensive range of STEC IMS solutions based on the key O-groups identified, but also emerging O-groups implicated in outbreaks and sporadic human diseases.

### Captivate™ O26

**CAP 003**

Magnetised particles, coated with antibodies specific to *Escherichia coli* O26.

### Captivate™ O111

**CAP 004**

Magnetised particles, coated with antibodies specific to *Escherichia coli* O111.

### Captivate™ O103

**CAP 005**

Magnetised particles, coated with antibodies specific to *Escherichia coli* O103.

### Captivate™ O145

**CAP 006**

Magnetised particles, coated with antibodies specific to *Escherichia coli* O145.

### Captivate™ O104

**CAP 007**

Magnetised particles, coated with antibodies specific to *Escherichia coli* O104.

### Captivate™ O121

**CAP 008**

Magnetised particles, coated with antibodies specific to *Escherichia coli* O121.

### Captivate™ O45

**CAP 009**

Magnetised particles, coated with antibodies specific to *Escherichia coli* O45.

### Captivate™ O91

**CAP 010**

Magnetised particles, coated with antibodies specific to *Escherichia coli* O91.

## *Captivate Accessories*

### Captivate™ Separator Rack

**CAP 100-12P**

Magnetic rack for use in the ***Captivate***™ isolation protocol. 12 tube capacity.

## *Custom Coating Service*

A coating service is available for coating our IMS reagent with alternative antibodies. Prices will be calculated on an individual basis.

For more information please contact our Technical Department

Tel: +44 (0) 161 820 3833

Fax: +44 (0) 161 820 5383

Email: info@labm.com

# 7. Pinnacle™ Pre-Poured Plates

The Pinnacle™ brand is a new line of ready-to-use plated culture medium, providing Lab M's high quality DCM prepared as ready-to-use plates.

Plates are poured under a stringent quality management system in a GMP environment and prepared in a clean room environment via a combination of different sized media preparators. Upon cooling plates are packed using a horizontal flow wrapping machine.

All plates are tested against a stringent quality system following ISO 11133:2014, which has been extended by Lab M to include batch-to-batch testing along with an enhanced panel of culture strains and more rigorous acceptance criteria.

All plates are stored prior to despatch in a dedicated cold room environment before shipping to customers upon QC release.

## Pinnacle™ LCA Listeria Chromogenic Agar (ISO)

**PIN001**

**Description**

Based on Lab M's established Harlequin™ Listeria Chromogenic Agar ISO (HAL010), according to the formulation of Ottaviani and Agosti), Pinnacle™ LCA is a ready-prepared selective medium for the isolation and presumptive identification of Listeria monocytogenes from foodstuffs and related materials as described in ISO 11290-1:1997.

Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Amphotericin B provide the medium's selectivity. Chromogenic activity is as a result of a chromogenic substrate for the detection of the β-glucosidase enzyme, common to all Listeria spp. and to a few strains of Enterococci and Bacilli.

The specific differential activity of this agar is obtained with a proprietary lecithin substrate for the detection of the phospholipase enzyme that will only be present in the L. monocytogenes colonies growing on this media. This enzyme activity will result in a halo of precipitation surrounding the target colonies.

With the combination of both the chromogenic and phospholipase enzyme reactions, it is possible to differentiate Listeria monocytogenes (blue colonies surrounded by an opaque halo) from other Listeria spp (blue colonies without an opaque halo).

| Typical Formula | g/litre |
|---|---|
| Meat Peptone | 18.0 |
| Tryptone | 6.0 |
| Yeast extract | 10.0 |
| Lithium chloride | 10.0 |
| Sodium chloride | 5.0 |
| Disodium hydrogen orthophosphate anhydrous | 2.5 |
| Sodium pyruvate | 2.0 |
| Glucose | 2.0 |
| Glycerophosphate | 1.0 |
| Magnesium sulphate | 0.5 |
| 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside | 0.05 |
| Agar | 13.5 |
| Deionised water | 1000ml |

Minimum Q.C. organisms:
*Listeria monocytogenes* WDCM 00021
*Escherichia coli* WDCM 00013 (Inhibited)

**Interpretation**

| organism | colony size (mm) | shape & surface | colour |
|---|---|---|---|
| *Listeria monocytogenes* | 1 - 2 | Round, Regular | Blue to blue-green, surrounded by opaque halo |
| *Listeria* spp. | 1 - 2 | Round, Regular | Blue to blue-green, without opaque halo |

Isolates presumptively identified as *Listeria* spp. and *Listeria monocytogenes* must be subjected to further biochemical tests to confirm their identity. Some strains of *Listeria ivanovii* may demonstrate lecithinase activity.

**References**

ISO 11290-1:1997 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Listeria monocytogenes* - Part 1: Detection method. Incorporating Amendment 1.

## Pinnacle™ TBGA (TBX)

**PIN002**

**Description**

A prepared medium based on Lab M's existing Harlequin™ Tryptone Bile Glucuronide Agar (TBGA), product reference HAL003. A medium developed for the simple enumeration of E. coli without the need for membranes, or pre-incubation on Minerals Modified Glutamate Medium. Based upon the formulation of Tryptone Bile Agar, LAB072, the medium has been modified by the addition of a chromogenic substrate to detect the β-glucuronidase enzyme, which is highly specific for E. coli*, and is detected by the MUG reagent in other formulations. The advantage of the chromogenic substrate is that it requires no UV lamp to visualise the reaction, and it is concentrated within the colony, facilitating easier enumeration in the presence of other organisms, or when large numbers are present on the plate.

| Typical Formula | g/litre |
|---|---|
| Tryptone | 20.0 |
| Bile Salts No.3 | 1.5 |
| X-glucuronide | 0.075 |
| Agar | 15.0 |
| Deionised water | 1000ml |

Minimum QC organisms: *Escherichia coli*
WDCM 00013 (blue/green)

**Inoculation:** Inoculate 0.5 ml of a 1:10 dilution of the sample and spread over the entire surface of the plate. Further dilution may be necessary if large numbers of *E. coli* are present, to ensure colonies can be easily counted.

**Incubation:** 30°C for 4 hours, followed by 18 hours at 44°C.

**Interpretation:** Count all blue/green colonies as presumptive *E. coli*, calculate the cfu/g in the original material. A simple indole test can be performed by placing one drop of Kovac's reagent onto a colony and if positive, a red halo will appear in the medium around the colony. If negative, then the halo will be white.

*96-97% of *E. coli* strains positive. A notable exception is *E. coli* 0157:H7.

**References**

Dibb, W.L. and Bottolfsen, K.L. (1984). Evaluation of Rosco Diagnostic ß-glucuronidase Tablets in the Identification of Urinary Isolates of *Escherichia coli*. Acta Path.Microbiol. Immunol. Scand. Sect. B 92 261-264.

Hansen, W. and Yourassowsky, E. (1984). Detection of ß-glucuronidase in Lactose Fermenting Members of the Family Enterobacteriaceae and its Presence in Bacterial Urine Cultures. J. Clin. Micro.20 (6) 1177-1179.

Robinson, B.J. (1984). Evaluation of a Fluorogenic Assay for Detection of *E. coli.* App & Env. Microbiol.48 (2) 285-288.

Perez, J.L., Berrocal, C.I. and Berrocal, L. (1986). Evaluation of a Commercial ß-glucuronidase Test for the Rapid and Economical Identification of *Escherichia coli*. J.App.Bacteriol. 61 541-545.

Raghubeer, E. and Matches, J.R. (1990). Temperature Range for Growth of *Escherichia coli* Serotype 0157:H7 and Selected Coliforms in *E. coli* Medium. J.Clin. Micro. 28 (4) 803-805.

Bolton, F.J. (1995) Personal Communication

## *Pinnacle™ CSIM (ISO)*

***Cronobacter sakazakii Isolation Medium (ISO)***

**PIN003**

**Description**

Cronobacter sakazakii (formerly Enterobacter sakazakii) is a member of the Enterobacteriaceae family and has been associated with serious outbreak infections in neonates (premature infants) which have been fed on infant formula milk. Although rarely causing infections in immunocompetent adults, C. sakazakii has been implicated in sepsis, meningitis and necrotising enterocolitis with a high death rate in neonates. This opportunistic pathogen is common in the environment and its ability to survive desiccation presents a significant risk for post pasteurisation contamination and survival in spray dried milk products.

C. sakazakii appears to constitutively express high levels of α-glucosidase. This enzyme hydrolyses the chromogenic substrate 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside present in the medium, producing green to blue-green coloured colonies. Other Enterobacteriaceae such as E. coli do not express strong α-glucosidase activity and appear colourless or purple due to the uptake of crystal violet.

The combination of sodium desoxycholate, crystal violet and elevated incubation temperature produce a very selective and specific medium. Non-Enterobacteriaceae may appear colourless or violet coloured (due to their inability to hydrolyse the chromogenic substrate) or are inhibited by the selective components and incubation temperature.
Using Lab M 's established Harlequin™ CSIM (ISO), this media formulation is currently recommended as part of the isolation protocol under ISO/TS 22964:2006(E) for the isolation of *Enterobacter sakazakii* from milk and milk products.

| Typical Formula | g/litre |
|---|---|
| Pancreatic peptone of casein | 7.0 |
| Yeast extract | 3.0 |
| Sodium chloride | 5.0 |
| Sodium desoxycholate | 0.6 |
| 5-bromo-4-chloro-3-indolyl-α-D-Glucoside | 0.15 |
| Crystal violet | 0.002 |
| Agar | 14.0 |
| Deionised Water | 1000mL |

Minimum Q.C. organisms:
*Cronobacter sakazakii* ATCC 12868
*Enterobacter aerogenes* WDCM 00175
*Bacillus cereus* WDCM 00001
*Staphylococcus aureus* WDCM 00034

**Inoculation:** Following selective enrichment in Modified Lauryl Sulphate Tryptose Broth Vancomycin Medium, streak onto HAL012 Harlequin™ *Cronobacter sakazakii* Isolation Medium (ISO).

**Incubation:** Incubate at 44°C + 0.5°C for 24 hours + 2 hours.

**Interpretation:** After incubation the plate should be assessed for typical colonies of *C. sakazakii*. Typical colonies are 1-3mm and are green to blue-green.

## *Pinnacle™ mLGA*

***(Membrane Lactose Glucuronide Agar)***

**PIN004**

**Description**

Pinnacle™ membrane Lactose Glucuronide Agar (mLGA) is a medium for the detection and identification of E. coli and coliforms from water samples.

Traditionally, membrane Lauryl Sulphate Broth (mLSB) has been used as the standard media for isolating coliforms (including E. coli) from water potentially contaminated with sewage. Harlequin™ membrane Lactose Glucuronide Agar (mLGA) is a modification of mLSB aimed at reducing costs by reducing the number of filters used per test sample and aiding in the recovery and identification of coliforms and E. coli. The medium has been modified from the mLSB formulation by the incorporation of X-glucuronide (BCIG), sodium pyruvate and agar.

X-glucuronide is a chromogenic substrate which detects the β-glucuronidase enzyme - highly specific for E. coli* - and allows for the presumptive isolation of E. coli. Sodium pyruvate aids the recovery of chlorine stressed organisms and agar is incorporated to remove the need for absorbent pads.

Using Lab M 's established Harlequin™ mLGA, this medium is recommended for the enumeration of coliform bacteria and E. coli by a single membrane filtration technique in The Microbiology of Drinking Water 2009 (previously Report 71).

*96-97% of E. coli strains positive. A notable exception is E. coli O157:H7.

| Typical Formula | g/litre |
|---|---|
| Peptone | 39.0 |
| Yeast Extract | 6.0 |
| Lactose | 30.0 |
| Phenol Red | 0.2 |
| Sodium Lauryl Sulphate | 1.0 |
| Sodium Pyruvate | 0.5 |
| X-Glucuronide | 0.2 |
| Agar | 10.0 |
| Deionised Water | 1000mL |

Minimum Q.C. organisms:
*Escherichia coli* WDCM 00013
*Enterobacter aerogenes* WDCM 00175
*Staphylococcus aureus* WDCM 00034 (inhibition)

**Inoculation:** *E. coli* and coliform counts can be performed on the same sample of water. The volume and dilution of test sample should be chosen so as the number of colonies on the membrane lies between 20 and 80. With waters expected to contain low numbers of coliforms, a sample of 100ml should be filtered. For full methodology refer to The Microbiology of Drinking Water 2002 section 4 B - The enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique.

**Incubation:** 4 hours at 30 °C followed by 14 hours at 37 °C

**Interpretation:** Count all green-blue colonies as presumptive *E. coli*, and all green-blue and yellow colonies as presumptive coliforms.

## *Pinnacle™ Salmonella ABC*

**PIN005**

**Description**

Pinnacle™ Salmonella ABC is a selective medium for the isolation of Salmonellae from food samples and is based on Lab M's established Harlequin™ Salmonella ABC.

Salmonella spp. can be differentiated from other members of the family Enterobacteriaceae by their ability to produce α-galactosidase in the absence of β-galactosidase. This medium utilises a dual chromogen system to visualise these enzyme activities. Salmonella ABC will also detect Salmonella typhi and paratyphi.

The first substrate, CHE-β-Gal, is enzymatically cleaved by ß-galactosidase producing organisms giving black colonies in the presence of iron. Most Enterobacteriaceae are β-galactosidase positive and these produce black colonies on Salmonella ABC. The second substrate, X-α-Gal, is hydrolysed by Salmonella spp. producing green colonies that are easily distinguished from the black or colourless colonies of other organisms. The medium is based on D.C.A Hynes and hence utilises sodium desoxycholate and sodium citrate as inhibitors. Isolation of Salmonella spp. by culture remains the most reliable method of detection. However, most media are highly non-specific and consequently place a heavy burden on the laboratory in terms of biochemical and serological confirmation of suspect colonies. With improved specificity, the ABC medium dramatically reduces the need for 'false positive' screening, saving labour and reducing consumable costs.

| Typical Formula | g/litre |
|---|---|
| Beef Extract | 5.0 |
| Peptone | 5.0 |
| Sodium citrate | 8.5 |
| Sodium desoxycholate | 5.0 |
| Agar | 12.0 |
| X-α-Gal | 0.08 |
| CHE-β-Gal | 0.3 |
| Ferric ammonium citrate | 0.5 |
| IPTG | 0.03 |
| Deionised Water | 1000mL |

Minimum QC organisms:
*Salmonella typhimurium* WDCM 00031
*Escherichia coli* WDCM 00013

**Growth Characteristics**

| organism | colony size (mm) | shape & surface | colour | other |
|---|---|---|---|---|
| *Salmonella* spp. | 1.5 - 3.5 | CV.E.G. | Green | (Black if β-galactosidase +ve) |
| *Shigella* spp. | 3.0 - 4.0 | CV.E.G. | Colourless | (Black if β-galactosidase +ve) |
| *E.coli* | PP - 2.5 | CV.E.G. | Black | (No Growth) |
| *Proteus* spp. | PP - 1.0 | CV.E.G. | Colourless | (Fishy Odour) |

**References**

Perry, J.D., Ford, M., Taylor, J., Jones, A., Freeman, R., Gould, F.K., (1999). ABC Medium, a New Chromogenic Agar for Selective Isolation of *Salmonella* spp. J. Clin. Micro. 37: 766-768.

## *Pinnacle™ Columbia Agar Base (25ml fill)*

**PIN006**

**Description**

A nutritious agar base capable of growing a wide variety of microorganisms. Lab M's Columbia Agar Base is available in a non-standard fill volume of 25ml and is suitable for use in a number of applications.

| Typical Formula | g/litre |
|---|---|
| Columbia Peptone Mixture | 25.1 |
| Soluble Starch | 1.0 |
| Sodium Chloride | 5.0 |
| Agar | 12.0 |
| Deionised Water | 1000mL |

Minimum Q.C. organisms:
*Pseudomonas aeruginosa* ATCC 9027
*Staphylococcus aureus* ATCC 6538
*Escherichia coli* ATCC 8739

## *Pinnacle™ Blood Agar No. 2 (25ml fill)*

**PIN007**

**Description**

A very rich agar base which, with the addition of blood, is capable of growing delicate clinical pathogens. The medium gives colonial appearances, haemolysis patterns and pigment production of diagnostic value. Lab M's Blood Agar No.2 is available in a non-standard fill volume of 25ml and is suitable for use in a number of applications.

| Typical Formula | g/litre |
|---|---|
| Tryptose | 15.0 |
| Soy Peptone | 2.5 |
| Yeast Extract | 5.0 |
| Sodium Chloride | 5.0 |
| Agar No. 2 | 12.0 |
| Deionised Water | 1000mL |

Minimum Q.C. organisms:
*Streptococcus pyogenes* NCTC 8198
*Escherichia coli* ATCC 25922
*Staphylococcus aureus* ATCC 25923
*Enterococcus faecalis* ATCC 29212
*Pseudomonas aeruiginosa* ATCC 27853

# *Pinnacle™ Legionella GVPC Medium (ISO)*

***(Glycine Vancomycin Polymixin Cycloheximide)***

**PIN008**

**Description**

Buffered Charcoal Yeast Extract (BCYE) medium is a basal agar for the isolation of *Legionella* species from water samples. It is based on the charcoal and yeast extract formulation of Feeley et al., with the addition of ACES (N-2-acetamido-2-aminoethanesulphonic acid) buffer and α-ketoglutarate to enhance performance, as described by Edelstein. Selective agents can be added to produce GVPC medium. This medium is recommended under ISO 11731:1998 for the isolation of *Legionella* species from water and conforms to the performance requirements of BS EN ISO 11133:2014.

Yeast extract provides a source of carbon and nitrogen, while activated charcoal acts as a detoxifying agent resulting in increased recovery from water samples. Iron (III) pyrophosphate provides iron necessary for the growth of *Legionella* species. ACES buffer and potassium hydroxide are used to maintain pH and improve recovery. L-cysteine, for which *Legionella* spp. are auxotrophic, and α-ketoglutarate, found to improve recovery of *Legionella* species, are also incorporated into the agar.

A number of other organisms commonly present in water samples are also able to grow well on basal BCYE medium. Therefore, a combination of selective agents is incorporated to produce glycine Vancomycin Polymyxin Cycloheximide medium, which is typically used for primary isolation of *Legionella* species. The mixture of glycine, vancomycin, and polymyxin B inhibits or suppresses most non-target bacterial species, both Gram-positive and Gram–negative, including common contaminants such as Enterococci, coliforms, and *Pseudomonas* species, while cycloheximide suppress the growth of yeasts and moulds.

| Typical Formula | g/litre |
|---|---|
| Yeast extract | 10.0 |
| Activated charcoal | 2.0 |
| ACES buffer | 10.0 |
| Potassium hydroxide | 2.8 |
| Iron (III) pyrophosphate | 0.25 |
| Agar | 14.0 |
| α-Ketoglutarate | 1.0 |
| L-Cysteine hydrochloride | 0.4 |
| Glycine | 3.0 |
| Vancomycin hydrochloride | 0.001 |
| Polymixin B sulphate | 80,000iu |
| Cycloheximide | 0.08 |
| Deionised water | 1000mL |

Minimum Q.C. organisms:
*Legionella pneumophila* WDCM 00107 or WDCM 00180
*Legionella anisa* WDCM 00106
*Pseudomonas aeruginosa* WDCM 00025 or WDCM 00026
*Escherichia coli* WDCM 00012 or WDCM 00013
*Enterococcus faecalis* WDCM 00009 or WDCM 00087

**Inoculation:** According to ISO 11731:1998, each concentrated water sample is tested after undergoing three protocols; Untreated, heat treatment and acid treatment. In all cases 0.1mL to 0.5mL of each sample portion is spread onto the agar surface with a sterile spreader.

**Incubation:** After insuring inocula has been absorbed invert the plates and incubate at 36±1°C for up to 10 days.

**Interpretation**

| Organism | shape & surface | Colour | Other |
|---|---|---|---|
| *Legionella spp.* | CV.E.G | Grey/white | Ground glass apperance |
| | | | Several species (including L. anisa & L. bozemanii) exhibit blue-white fluorescence under long-wave UV light |

Note - *Legionella* colonies are often grey/white in colour but can exhibit a wide range of colours.

**References**


Feeley, J.C., Gibson, R.J. et al. (1979). Journal of Clinical Microbiology 10: 437-441

Pesculle, A.H., Feeley, J.C. et al. (1980). Journal of Infectious Disease 141: 727-732

Edelstein, P.H. (1981). Journal of Clinical Microbiology 14: 298-303

BS EN ISO 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media

ISO 11731:1998 Water quality –Detection and enumeration of Legionella

ISO 11731-2:2004 Water quality – Detection and enumeration of Legionella – Part 2: Direct membrane filtration method for waters with low bacterial counts


# *Pinnacle™ Legionella BCYE Medium (ISO)*

***(Buffered Charcoal Yeast Extract)***

**PIN009**

**Description**

Buffered Charcoal Yeast Extract (BCYE) medium is a basal agar for the isolation of *Legionella* species from water samples. It is based on the charcoal and yeast extract formulation of Feeley et al., with the addition of ACES (N-2-acetamido-2-aminoethanesulphonic acid) buffer and α-ketoglutarate to enhance performance, as described by Edelstein. This medium is recommended under ISO 11731:1998 for the isolation of *Legionella* species from water and conforms to the performance requirements of BS EN ISO 11133:2014.

Yeast extract provides a source of carbon and nitrogen, while activated charcoal acts as a detoxifying agent resulting in increased recovery from water samples. Iron (III) pyrophosphate provides iron necessary for the growth of *Legionella* species. ACES buffer and potassium hydroxide are used to maintain pH and improve recovery. L-cysteine, for which *Legionella* spp. are auxotrophic, and α-ketoglutarate, found to improve recovery of *Legionella* species, are also incorporated into the agar.

| Typical Formula | g/litre |
|---|---|
| Yeast extract | 10.0 |
| Activated charcoal | 2.0 |
| ACES buffer | 10.0 |
| Potassium hydroxide | 2.8 |
| Iron (III) pyrophosphate | 0.25 |
| Agar | 14.0 |
| α-Ketoglutarate | 1.0 |
| L-Cysteine hydrochloride | 0.4 |
| Deionised water | 1000mL |

Minimum Q.C. organisms:
*Legionella pneumophila* WDCM 00107 or WDCM 00180
*Legionella anisa* WDCM 00106

**Inoculation:** According to ISO 11731:1998, presumptive *Legionella* colonies from GVPC agar plates are subcultured onto BCYE for further confirmation.

**Incubation:** Incubate at 36±1°C for at least 2 days

**Interpretation**

| Organism | shape & surface | Colour | Other |
|---|---|---|---|
| *Legionella spp.* | CV.E.G | Grey/white | Ground glass apperance Several species (including L. anisa & L. bozemanii) exhibit blue-white fluorescence under long-wave UV light |

Note - *Legionella* colonies are often grey/white in colour but can exhibit a wide range of colours.

**References**

Feeley, J.C., Gibson, R.J. et al. (1979). Journal of Clinical Microbiology 10: 437-441

Pesculle, A.H., Feeley, J.C. et al. (1980). Journal of Infectious Disease 141: 727-732

Edelstein, P.H. (1981). Journal of Clinical Microbiology 14: 298-303

BS EN ISO 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media

ISO 11731:1998 Water quality –Detection and enumeration of Legionella

ISO 11731-2:2004 Water quality – Detection and enumeration of Legionella – Part 2: Direct membrane filtration method for waters with low bacterial counts

# 8. Lyophilised Media Supplements

**Presentation and Shelf Life**

**Lab M** lyophilised supplements are presented in packs of 10 vials, and for the majority of the supplements each vial is sufficient for 500ml of medium. Larger and smaller volumes are indicated for relevant products.

The shelf life of freeze-dried supplements is 2-3 years provided they are stored in a refrigerator at 2-8˚C. The shelf life of each product is detailed on the individual product literature and labelling. Once rehydrated the stability of antibiotics varies greatly and will determine the shelf life of the prepared agars and broths. For this reason any unused, rehydrated, supplement should be discarded, as even deep-freezing may not prevent the rapid degradation of the antibiotics. To ensure the correct level of selective supplements the entire vial contents must be added to the stated volume of cooled, molten medium.

**Rehydration**

Vials should be rehydrated aseptically using a pipette charged with the appropriate volume of the specified diluent for the particular supplement being added. The supplement should be rehydrated, withdrawn and added to the medium in a single process, followed by immediate disposal of the pipette into an approved container.

**Addition**

Most antibiotics are heat labile, and so to prevent a reduction of potency the medium should be cooled to 47-50˚C (as specified for each individual product), by holding in a water bath set at this temperature.

Once the supplement has been added the medium must be gently but thoroughly mixed to ensure that the selective agents are evenly distributed. Failure to do this will result in a range of concentrations in the plates/bottles and consequent inconsistency in results. Media should not be held for an additional period at 47-50˚C, but poured immediately. The shelf life of supplemented media is governed by the stability of the added components, and is generally shorter than unsupplemented agars and broths. For information on the shelf life of prepared media consult the individual product listings in the previous section of the manual.

## *Chloramphenicol Supplement*

**X009**

**CHLORAMPHENICOL for the selective isolation of yeasts and moulds from food, environmental and clinical specimens.**

For larger volumes X209 is available (1 vial per 1L)

Chloramphenicol's broad antibiotic spectrum suppresses most contaminating bacteria allowing the yeasts and moulds to grow. It can be added to such media as LAB009 Sabouraud Dextrose Agar, LAB036 Rose Bengal Chloramphenicol Agar, LAB037 Malt Extract Agar and LAB117 Dermatophyte Test Medium to increase their selectivity whilst not lowering the pH. Reduction of pH will increase the selectivity of a yeast and mould medium but will also inhibit some yeasts as well as having a deleterious effect on the agar gel.

| Final Concentration | mg/litre |
|---|---|
| Chloramphenicol | 100 |
| Add 1 vial X009 to 500ml medium | |

Rehydrate contents of vial with 5ml of Ethyl or Methyl alcohol. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**References:**

Jervis, B. (1973). Rose Bengal Chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in foods. J. Appl. Bact. 36 Pages 723-727.

## *Colistin & Nalidixic Acid Selective Supplement*

**X011**

**COLISTIN, NALIDIXIC ACID for the isolation of *G. vaginalis* from clinical material.**

Suitable for addition to LAB001 Columbia Agar or LAB015 Blood Agar Base No. 2 to produce a selective isolation medium.

| Final Concentration | mg/litre |
|---|---|
| Colistin | 10 |
| Nalidixic acid | 15 |
| Add 1 vial X011 to 500ml medium | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, together with any other additives, mix gently and pour.

**Reference:**

Goldberg, R.L., Washington, J.A. II (1976). "Comparison of Isolation of *Haemophilus vaginalis (Corynebacterium vaginalae)* from Peptone-Starch-Dextrose Agar and Columbia, Colistin, Nalidixic Acid Agar. J. Clin. Microbiol. 4(3): 245.

## *Colistin & Nalidixic Acid*

**X012**

**COLISTIN, NALIDIXIC ACID for the preparation of Columbia C.N.A. medium.**

A medium selective for Gram positive cocci is obtained when this antibiotic mixture is added to LAB001 Columbia Agar.

| Final Concentration | mg/litre |
|---|---|
| Colistin | 10 |
| Nalidixic acid | 10 |
| Add 1 vial X012 to 500ml medium | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, together with any other additives, mix gently and pour.

**Reference:**

Ellner, P.D., Stossel, C.I., Drakeford, E., Vasi, F. (1966). "A new culture medium for medical bacteriology." Amer. J. Clin. Path. 45: 502.

## *Colistin & Oxolinic Acid*

**X013**

**COLISTIN, OXOLINIC ACID for the selective isolation of streptococci from clinical material.**

When added to LAB001 Columbia agar or LAB015 Blood Agar Base No. 2, X013 renders the medium selective for streptococci. Alteration in haemolysis patterns may occur when azide or crystal violet are employed as selective agents but this does not occur with X013.

| Final Concentration | mg/litre |
|---|---|
| Colistin | 10 |
| Oxolinic acid | 5 |
| Add 1 vial X013 to 500ml medium | |

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

**Reference:**

Petts, D. (1984). Colistin - Oxolinic Acid - Blood Agar: a new selective medium for streptococci. J. Clin. Microbiol. 19: 4-7.

## *Neomycin 75mg*

**X015**

**NEOMYCIN 75 for the isolation of *Clostridium* spp. and other anaerobes.**

When added to blood agar the resulting medium will allow the growth of clostridia, most *Bacteriodes fragilis* strains and some anaerobic cocci.

| Final Concentration | mg/litre |
|---|---|
| Neomycin | 75 |
| Add 1 vial X015 to 500ml medium | |

Reconstitute each vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

## *Neomycin 100mg*

**X016**

**NEOMYCIN 100 for the selective isolation of *Clostridium* spp.**

When added to egg yolk medium this supplement will allow the growth of clostridia whilst inhibiting other lecithinase producing organisms.

| Final Concentration | mg/litre |
|---|---|
| Neomycin | 100 |
| Add 1 vial X016 to 500ml medium | |

Reconstitute each vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

## *Kanamycin 75mg*

**X018**

**KANAMYCIN 75 for the selective isolation of *Clostridium* spp. and other anaerobes.**

An alternative to X015. Kanamycin is more inhibitory to anaerobic cocci.

| Final Concentration | mg/litre |
|---|---|
| Kanamycin | 75 |

Add 1 vial X018 to 500ml medium
Reconstitution as X015, X016.

**References:**


Lowbury, C.J.L., Lilly, H.A. (1955). A selective plate medium for *Cl. welchi.* J. Path. & Bact. 70: 105.

Collee, J.G., Watt, B. (1971). Changing approaches to the sporing anaerobes in medical microbiology. Spore Research ed. A. N. Barkeer.

Sutter, V.L., Citron, D.M., Edelstein, M.A.C., Finegold, S.M. (1985). Wadsworth Anaerobic Bacteriology Manual 4 ed. Star publishers, Belmont, California.

Wren, M.W.D. (1980). Multiple selective media for the isolation of anaerobic bacteria from clinical specimens. J. Clin. Path. 33: 61-65


## *P-INC Supplement (PNVC)*

**X019**

**PENICILLIN, NISIN, CRYSTAL VIOLET, for accelerated shelf life determination of dairy products.**

For larger volumes X219 is available (1 vial per 1L)

The Pre-incubation test uses a selective mixture to inhibit Gram positive organisms whilst allowing the growth of Gram negative bacteria, the main cause of post-pasteurisation contamination and a major factor in determining the shelf life of the product. The technique is also useful for monitoring plant hygiene.

| Final Concentration | mg/litre |
|---|---|
| Penicillin | 20,000iu/litre |
| Nisin | 40,000iu/litre m |
| Crystal violet | 2.0 |
| Add 1 vial of X019 to 200ml of Milk Agar LAB019 | |

Rehydrate contents of 1 vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix thoroughly and pour plates.

**Method A**

Pre-incubate test material at 21˚C for 24hr. Prepare suitable dilution series, and inoculate Milk Agar plates containing P-INC supplement. Incubate at 21˚C for 24hr, and count all colonies (some may be small, use of a hand lens is recommended). Calculate the CFU/ml and using the tables of Griffith's *et al* the shelf life can be determined.

**Method B**

Rehydrate X219 with 1ml of deionised water only, add 0.1ml to the test material and incubate at 20˚C for 24hr. Prepare suitable dilution series, and inoculate Milk Agar plates. Proceed as for Method A above.

## *VCNT Selective Supplement*

**X068**

**V.C.N.T. VANCOMYCIN, COLISTIN, NYSTATIN, TRIMETHOPRIM for Thayer Martin Medium.**

The addition of trimethoprim in V.C.N.T. inhibits the swarming of *Proteus* spp. which occasionally make interpretation difficult.

| Final Concentration | mg/litre |
|---|---|
| Vancomycin | 3 |
| Colistin | 7.5 |
| Nystatin | 12.5 |
| Trimethoprim | 5 |
| Add 1 vial X068 to 500ml medium | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47°C together with other additives, mix gently and pour.

**Reference:**


Thayer, J.D. and Martin, J.E. (1966). Improved medium selective for the cultivation of *N. gonorrhoeae* and *N. meningitidis.* Public Health rep. 81: 559-562.

## *LCAT Selective Supplement*

**X070**

**L.C.A.T. LINCOMYCIN, COLISTIN, AMPHOTERICIN, TRIMETHOPRIM for the isolation of *Neisseria* spp. from clinical material.**

L.C.A.T. is often preferred to X068 V.C.N.T. for the isolation of *N. gonorrhoeae* because of the emergence of vancomycin sensitive strains. The antifungal agent amphotericin is more readily soluble and therefore a more active antifungal than nystatin. L.C.A.T. is quoted as the selective agent for New York City G.C. agar but can readily be substituted for V.C.N. or V.C.N.T. in Thayer Martin G.C. agar.

| Final Concentration | mg/litre |
|---|---|
| Lincomycin | 1 |
| Colistin | 6 |
| Amphotericin | 1 |
| Trimethoprim | 6.5 |
| Add 1 vial X070 to 500ml medium | |

Rehydrate contents of vial with 5ml sterile 25% alcohol in water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

**Reference:**

Young, H. (1978). Cultural Diagnosis of Gonorrhoea with modified N.Y.C. Medium. Brit. Journ. Ven. Dis. 54: 36-40.

## *Polymyxin & Ceftazidime Selective Supplement*

**X072**

**POLYMYXIN B, CEFTAZIDIME supplement for the isolation of *Listeria monocytogenes*.**

For addition to LAB172, LMBA

| Final Concentration | mg/litre |
|---|---|
| Polymyxin B | 10 |
| Ceftazidime | 20 |
| Add 1 vial X072 and 1 vial of X072N to 500ml medium. | |

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

## *Nalidixic Acid Selective Supplement*

**X072N**

**NALIDIXIC ACID supplement for the isolation of *Listeria monocytogenes*.**

For addition to LAB172, LMBA

| Final Concentration | mg/litre |
|---|---|
| Nalidixic acid | 40 |
| Add 1 vial X072N and 1 vial of X072 to 500ml medium. | |

Rehydrate contents of vial by the addition of 5 ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

## *Polymyxin B*

**X074**

**POLYMYXIN for the isolation of *B. cereus* from foods.**

For larger volumes X274 is available (1 vial per 2L)

Suitable for the preparation of LAB073 Bacillus cereus Medium (P.R.E.P.). The addition of X073 sterile egg yolk emulsion is also required.

| Final Concentration | |
|---|---|
| Polymyxin B | 8mg/litre = 64,000i.u/litre |
| Add 1 vial X074 to 500ml medium | |

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with egg yolk emulsion, mix gently and pour.

**Reference:**

Micro-organisms in Food. Ed. Thatcher, F.S., Clarke, D.F. published by Univ. of Toronto Press.

## *RPF Supplement*

**X086**

**RPF Supplement**
**Rabbit Plasma Fibrinogen Supplement**

**Description**

Bovine fibrinogen, rabbit plasma, trypsin inhibitor and potassium tellurite for the isolation of *Staphylococcus aureus*.

For addition to LAB285 Baird-Parker Medium Base (ISO) and LAB085 Baird-Parker Medium Base.

| Final Concentration | amount / vial | amount / litre |
|---|---|---|
| Bovine Fibrinogen | 0.375g | 3.75g |
| Rabbit Plasma | 2.5ml | 25ml |
| Trypsin Inhibitor | 2.5mg | 25mg |
| Potassium Tellurite | 2.5mg | 25mg |

Add 1 vial of X086 to 90mL of medium.

Rehydrate contents of vial by the addition of 10mL sterile deionised water. Add aseptically to sterilised media (cooled to 47°C), mix gently to evenly distribute and pour.

## *Oxytetracycline Supplement*

**X089**

**OXYTETRACYCLINE for O.G.Y.E. medium.**

For use with LAB089 Oxytetracycline Glucose Yeast Extract Agar for the enumeration of yeasts and moulds from foodstuffs. Highly proteinaceous foods and incubation above 30˚C will inactivate oxytetracycline.

| Final Concentration | mg/litre |
|---|---|
| Oxytetracycline | 100 |
| Add 1 vial X089 to 500ml medium | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**References:**

Mossel, D.A.A., *et al*. (1970). O.G.Y.E. for the selective enumeration of moulds and yeasts in food and clinical material. J. Appl. Bact. 35: 454-457.

## *Nalidixic Acid & Vancomycin*

**X090**

**NALIDIXIC ACID, VANCOMYCIN for the isolation of Gram negative anaerobes from clinical material.**

Suitable for use with LAB090 Fastidious Anaerobe Agar. When used with other blood agar bases, e.g. LAB001 Columbia Agar, further enrichment of the medium with haemin and menadione is beneficial.

| Final Concentration | mg/litre |
|---|---|
| Nalidixic acid | 10 |
| Vancomycin | 2.5 |
| Add 1 vial X090 to 500ml medium | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

**Reference:**

Wren, M.W.D., 1980. J. Clin. Path. 33: 61-65. Multiple Selective Media for the isolation of anaerobic bacteria.

## *Cycloserine & Cefoxitin*

**X093**

**CYCLOSERINE, CEFOXITIN for the isolation of *Clostridium difficile* from clinical materials.**

Suitable for use with LAB090 Fastidious Anaerobe Agar.

| Final Concentration | mg/litre |
|---|---|
| D-Cycloserine | 250 |
| Cefoxitin | 8 |
| Add 1 vial X093 to 500ml medium | |

Rehydrate contents of vial with 5ml of water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

**Reference:**

George, W.L., Sutter, V.L., Citron, D., Finegold, S.M. (1976). Selective and differential medium for isolation of *Clostridium difficile.*

## *CN Supplement*

**X107**

**C.N. CETRIMIDE, NALIDIXIC ACID for the isolation of *Pseudomonas aeruginosa.***

Suitable for use with LAB108 Pseudomonas Agar to make the medium selective for *Ps. aeruginosa.*

| Final Concentration | mg/litre |
|---|---|
| Cetrimide | 200 |
| Nalidixic acid | 15 |
| Add 1 vial X107 to 500ml medium | |

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**Reference:**

Goto, S., Enomoto, S. 1970. Jap. J. Microbiol. 14: 65-72.

## *CFC Supplement*

**X108**

**MODIFIED C.F.C. – CEPHALOTHIN, FUCIDIN, CETRIMIDE for the selective isolation of *Pseudomonas* spp.**

When added to LAB108 Pseudomonas Agar, to prepare C.F.C. medium this supplement can be used to select pseudomonads from food and environmental samples.

For larger volumes X223 is available (1 vial per 2L)

| Final Concentration | mg/litre |
|---|---|
| Cephalothin | 50 |
| Fucidin | 10 |
| Cetrimide | 10 |
| Add 1 vial X108 to 500ml medium | |

Rehydrate contents of vial with 5ml of sterile 50% alcohol. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**Reference:**

Mead, G.C. and Adams, B.W. (1977). Br. Poult. Sci. 18: 661-667

## *Sulphadiazine Supplement*

**X109**

**SULPHADIAZINE. SEE ALSO X110**

For use with LAB109 Perfringens agar to prepare O.P.S.P. for the selective isolation of *Clostridium perfringens* from foodstuffs.

| Final Concentration | mg/litre |
|---|---|
| Sulphadiazine | 100 |
| Add 1 vial X109 and 1 vial X110 to 500ml medium | |

Rehydrate contents of vials with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**Reference:**

Handford, P.M. (1974). J. Appl. Bact. 37, 559-570.

## *Oleandomycin & Polymyxin Supplement*

**X110**

**OLEANDOMYCIN PHOSPHATE, POLYMYXIN. SEE ALSO X109**

For use with LAB109 Perfringens agar to prepare O.P.S.P. for the selective isolation of *Clostridium perfringens* from foodstuffs.

| Final Concentration | mg/litre |
|---|---|
| Oleandomycin | 0.5 |
| Polymyxin | 10,000 i.u./litre |
| Add 1 vial X109 and 1 vial X110 to 500ml medium | |

Rehydrate contents of vials with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**Reference:**

Handford, P.M. (1974). J. Appl. Bact. 37, 559-570.

## *Cefoperazone & Amphotericin Selective Supplement*

**X112**

**CEFOPERAZONE, AMPHOTERICIN for the isolation of *Campylobacter* spp. from clinical, environmental and food samples.**

For larger volumes X212 is available (1 vial per 1L)

Suitable for use with LAB112 Campylobacter Selective Medium (blood free) or with blood agar media. Incubation at 37˚C gives better results than at 42˚C and is generally more convenient.

| Final Concentration | mg/litre |
|---|---|
| Cefoperazone | 32 |
| Amphotericin | 10 |
| Add 1 vial X112 to 500ml medium | |

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**Reference:**

Bolton, F.J., Hutchinson, D.N., Parker, G. (1988). Reassessment of Selective Agars and Filtration Techniques for Isolation of Campylobacter Species from Faeces. Eur. J. Clin. Microbiol. Infect. Dis. 7: 155-160.

## *Modified Preston Campylobacter Supplement*

**X114**

**Modified Preston Campylobacter Supplement for the isolation of *Campylobacter* spp.**

For use with LAB015 Blood Agar Base supplemented with 5% lysed horse blood or LAB014 Nutrient Broth No.2 supplemented with 5% lysed horse blood and X115 Campylobacter Growth Supplement.

| Final Concentration | Per vial | Conc. in medium |
|---|---|---|
| Rifampicin | 5mg | 10mg/L |
| Polymyxin B | 2500 IU | 5000 IU |
| Trimethoprim | 5mg | 10mg/L |
| Amphotericin B | 5mg | 10mg/L |

**Rehydration**

Wearing latex gloves aseptically reconstitute the contents of the vial with 5ml of sterile 50% ethanol using a sterile pipette. Ensure contents of vial are well mixed before addition to culture media.

## *Campylobacter Growth Supplement*

**X115**

**Campylobacter growth supplement for the isolation of Camylobacter spp.**

Suitable for use with LAB014 Modified Preston Campylobacter Medium (Nutrient Broth No. 2)

| Formulation | Per vial | Conc. in medium (LAB014) |
|---|---|---|
| Sodium pyruvate | 0.125 g | 0.25 g |
| Sodium metabisulphite | 0.125 g | 0.25 g |
| Ferrous sulphate | 0.125 g | 0.25 g |

Wearing latex gloves aseptically reconstitute the contents of the vial with 5 ml of sterile deionised water using a sterile pipette.

## *CIN Selective Supplement*

**X120**

**C.I.N. - CEFSULODIN, IRGASAN, NOVOBIOCIN for the isolation of *Yersinia* spp. from clinical and environmental material.**

For addition to LAB120 Yersinia C.I.N. Agar Base used in the selective isolation of *Y. enterocolitica.*

| Final Concentration | mg/litre |
|---|---|
| Cefsulodin | 15 |
| Irgasan | 4 |
| Novobiocin | 2.5 |
| Add 1 vial X120 to 500ml medium | |

Rehydrate contents of vial with 5ml of 30% sterile alcohol. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**References:**

Schiemann, D.A. (1979). Synthesis of a selective medium of Yersinia enterocolitica. Can. J. Microbiol. 25 (2): 1298.

Schiemann, D.A. (1980). Isolation of toxigenic Yersinia enterocolitica from retail pork products. J. Food Prot. 43: 360.

Schiemann, D.A. (1982). Development of a two-step enrichment procedure for recovery of Yersinia enterocolitica from food. Appl. Microbiol. 43 (1): 14.

## *CNCAF Selective Supplement*

**X123**

**Cefotetan, Natamycin, Colistin, Acriflavine, Fosfomycin (CNCAF) Selective Supplement**

**Description**

For the isolation of *Listeria monocytogenes* from environmental, clinical and food samples. This supplement may be added to LAB122 Listeria Isolation Medium Oxford, LAB206 Listeria Isolation Media (Oxford).

| Formulation | Per vial | Concentration in medium LAB122 / LAB206 |
|---|---|---|
| Cefotetan | 1 mg | 2 mg/L |
| Natamycin | 12.5 mg | 25 mg/L |
| Colistin | 10 mg | 20 mg/L |
| Acriflavine | 2.5 mg | 5 mg/L |
| Fosfomycin | 5 mg | 10 mg/L |
| **Vials per litre of medium** | | **2** |

**Appearance**

White to off-white lyophilised tablet.

**Reconstitution**

Contents of the vial should be reconstituted by the addition of sterile 50% ethanol in water. Add aseptically to sterilised medium cooled to 47°C. Mix gently then pour.

## *CVTN Supplement*

**X132**

**Cefoperazone, Vancomycin, Trimethoprim, Natamycin (CVTN) Selective Supplement**

**Description**

For the isolation of *Campylobacter* spp. from food and environmental samples by the enrichment broth technique. Developed for use with LAB135 Campylobacter Enrichment Broth. Gives higher isolation rates than Preston Broth and does not require modified atmosphere incubation.

| Formulation | Per vial | Conc. in medium (LAB135) |
|---|---|---|
| Cefoperazone | 10mg | 20mg/L |
| Vancomycin | 10mg | 20mg/L |
| Trimethoprim | 10mg | 20mg/L |
| Natamycin | 12.5mg | 25mg/L |
| **Vials per litre of medium** | | **2** |

**Appearance**

White to off-white lyophilised tablet.

**Reconstitution**

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47ºC. Mix gently and dispense into sterile containers.

## *NAN Selective Supplement*

**X139**

**N.A.N. NALIDIXIC ACID, ACRIFLAVINE, NATAMYCIN.**

An alternative natamycin based supplement for the selective enrichment broth culture of *Listeria* spp.

For addition to LAB 138, *Listeria* Enrichment Broth and LAB139, Buffered *Listeria* Enrichment Broth.

| Final Concentration | mg/litre |
|---|---|
| Nalidixic acid | 40 |
| Acriflavine | 15 |
| Natamycin | 25 |
| Add 1 vial of X139 to 500ml medium. | |

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and dispense.

## *Cepacia Selective Supplement*

**X140**

**TICARCILLIN, POLYMYXIN, for the isolation of *Burkholderia (Pseudomonas) cepacia***

Suitable for use with LAB108 pseudomonas selective agar, or specific selective bases such as that described by Gilligan *et al.*

| Final Concentration | mg/litre |
|---|---|
| Ticarcillin | 100 |
| Polymyxin | 300,000 iu/litre |
| Add 1 vial to 500ml of medium | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

**Reference:**

Gilligan, P.H., Gage, P.A., Bradshaw, L.M., Schidlow, D.V., DeCicco, B.T. (1985) Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis. J.Clin.Microbiol. 22 (1) 5-8.

## *PAC Selective Supplement*

**X144**

**P.A.C. supplement for the enrichment and isolation of *Listeria* spp from food and environmental samples.**

For the addition to LAB144 Palcam Broth and Lab 148 Palcam Agar

| Final concentration | mg/litre |
|---|---|
| Polymyxin | 10 |
| Acriflavine | 5 |
| Ceftazidime | 20 |
| Add 1 vial of X144 to 500ml of Palcam Broth or Agar | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, along with other additives, mix well and pour.

## *Novobiocin*

**X150**

**NOVOBIOCIN for the enrichment of *E. coli* O157:H7 from food, environmental and clinical samples.**

For the addition to LAB165 O157 Broth MTSB

| Final concentration | mg/litre |
|---|---|
| Novobiocin | 20 |
| Add 1 vial of X150 to 500ml of O157 Broth MTSB. | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

## *UVM I Supplement*

**X155**

**UVMI. Supplement for the primary enrichment of *Listeria* spp from food and environmental samples.**

For addition to LAB 155 UVM Broth Base

| Final Concentration | mg/litre |
|---|---|
| Nalidixic acid | 20 |
| Acriflavine | 12 |
| Add 1 vial of X155 to 500ml of UVM Broth Base | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

## *Cefixime Tellurite Supplement*

**X161**

**CEFIXIME TELLURITE supplement for the isolation of *E. coli* O157:H7 from food, environmental and clinical samples.**

For the addition to LAB161 Sorbitol MacConkey Agar (SMAC) or HAL006 (BCIG-SMAC).

| Final concentration | mg/litre |
|---|---|
| Cefixime | 0.05 |
| Potassium tellurite | 2.5 |
| Add 1 vial of X161 to 500 ml of Sorbitol MacConkey Agar (SMAC) or HAL006 (BCIG-SMAC) | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

## *Half Fraser Supplement*

**X164**

**HALF FRASER supplement for the primary enrichment of *Listeria* spp from food and environmental samples.**

**For larger volumes X564 is available (1 vial per 2.25 L)**

For addition to LAB164 Fraser Broth Base

| Final Concentration | mg/litre |
|---|---|
| Ferric ammonium citrate | 500 |
| Acriflavine | 12.5 |
| Nalidixic acid | 10 |
| Add 1 vial of X164 to 450ml of Fraser Broth Base | |
| Add 1 vial of X564 to 2.25 litres of Fraser Broth Base | |

Rehydrate contents of vial with 2ml 50% methanol (5ml for X564). Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

## *Fraser Supplement*

**X165**

**FRASER supplement for the secondary enrichment of *Listeria* spp from food and environmental samples.**

For addition to LAB164 Fraser Broth Base

| Final Concentration | mg/litre |
|---|---|
| Ferric ammonium citrate | 500 |
| Acriflavine | 25 |
| Nalidixic acid | 20 |
| Add 1 vial of X165 to 500ml of Fraser Broth Base | |

Rehydrate contents of vial with 2ml 50% methanol. Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

## *ORSIM Selective Supplement*

**X192**

**OXACILLIN, POLYMYXIN B supplement for the isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA).**

For addition to LAB192, ORSIM (Oxcacillin Resistant *Staphylococcus* Isolation Medium).

| Final Concentration | mg/litre |
|---|---|
| Oxacillin | 2 |
| Polymyxin B | 25,000 I.U |
| Add 1 vial of X192 to 500ml medium. | |

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and dispense.

## *Polymyxin B*

**X193**

**POLYMYXIN B for the isolation of *Bacillus cereus* from foods.**

The addition of X073, Egg Yolk Emulsion, is also required. For addition to LAB193, PEMBA *Bacillus cereus* Medium.

| Final Concentration | mg/litre |
|---|---|
| Polymyxin B | 100,000 IU |
| Add 1 vial X193 to 500ml medium | |

Rehydrate contents of vial by the addition of 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

## *D-Cycloserine Supplement*

**X194**

**D-CYCLOSERINE supplement for the isolation of *Clostridium perfringens* from foods.**

For use with LAB194, Perfringens Agar Base (TSC).

| Final Concentration | mg/litre |
|---|---|
| D-Cycloserine | 400 |
| Add 1 vial of X194 to 500mls medium. | |

Rehydrate contents of vial by the addition of 5mls of sterile deionised water. Add aseptically to sterilised medium cooled to 47°C, mix gently and pour.

## *GVPC Selective Supplement*

**X195**

**GVPC Selective Supplement - Glycine, Vancomycin, Polymyxin B, Cycloheximide**

**Description**

A selective supplement developed for use with LAB195 BCYE Legionella Isolation Medium for the isolation of *Legionella* spp. X195 GVPC Supplement should be used in conjunction with X196 BCYE Growth Supplement.

| Formulation | Per vial | Conc. in medium (LAB195) |
|---|---|---|
| Glycine | 1500mg | 3000mg/L |
| Vancomycin hydrochloride | 0.5mg | 1mg/L |
| Polymyxin B sulphate | 39600 IU | 79200 IU/L |
| Cycloheximide | 40mg | 80mg/L |
| **Vials per litre of medium** | | **2** |

**Appearance**

Off-white lyophilised tablet.

**Hazard classification**

T - Toxic

**Reconstitution**

Rehydrate contents of vial with 10ml sterile deionised water. Add aseptically to sterilised medium cooled to 47ºC. Mix gently and dispense into sterile containers.

**References**

International Standard. ISO 11731:1998(E). Water quality- Detection and enumeration of Legionella

## *BCYE Growth Supplement*

**X196**

**BCYE Growth Supplement**

**Description**

L-Cysteine Hydrochloride and α-Ketoglutarate BCYE growth supplement for the isolation of *Legionella* spp. For addition to LAB195 BCYE Legionella Isolation Medium

| Formulation | mg/litre |
|---|---|
| L-Cysteine Hydrochloride | 400 |
| α-Ketoglutarate | 1000 |
| Add 1 vial per 500mL of sterilised medium as appropriate. | |

**References**

ISO 11731:1998(E) Water quality- Detection and enumeration of Legionella

## *BCYE Growth Supplement (no L-Cysteine)*

**X197**

**BCYE Growth Supplement (no L-cysteine)**

**Description**

α-Ketoglutarate growth supplement for the presumptive identification of *Legionella* spp. For addition to LAB195 BCYE Legionella Isolation Medium.

| Formulation | mg/litre |
|---|---|
| α-Ketoglutarate | 1000 |
| Add 1 vial per 500mL of sterilised medium as appropriate. | |

**References**

ISO 11731:1998(E) Water quality- Detection and enumeration of Legionella

## *Chloramphenicol Supplement*

**X209**

**CHLORAMPHENICOL for the selective isolation of yeasts and moulds from food, environmental and clinical specimens.**

For smaller volumes X009 is available (1 vial per 500ml)

Chloramphenicol's broad antibiotic spectrum suppresses most contaminating bacteria allowing the yeasts and moulds to grow. It can be added to such media as LAB009 Sabouraud Dextrose Agar, LAB036 Rose Bengal Chloramphenicol Agar, LAB037 Malt Extract Agar and LAB117 Dermatophyte Test Medium to increase their selectivity whilst not lowering the pH. Reduction of pH will increase the selectivity of a yeast and mould medium but will also inhibit some yeasts as well as having a deleterious effect on the agar gel.

| Final Concentration | mg/litre |
|---|---|
| Chloramphenicol | 100 |
| Add 1 vial X209 to 1 litre medium | |

Rehydrate contents of vial with 5ml of Ethyl or Methyl alcohol. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**References:**

Jervis, B. (1973). Rose Bengal Chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in foods. J. Appl. Bact. 36 Pages 723-727.

## *Cefoperazone & Amphotericin Selective Supplement*

**X212**

**CEFOPERAZONE, AMPHOTERICIN for the isolation of *Campylobacter* spp. from clinical, environmental and food samples.**

For smaller volumes X112 is available (1 vial per 500ml)

Suitable for use with LAB112 Campylobacter Selective Medium (blood free) or with blood agar media. Incubation at 37˚C gives better results than at 42˚C and is generally more convenient.

| Final Concentration | mg/litre |
|---|---|
| Cefoperazone | 32 |
| Amphotericin | 10 |
| Add 1 vial X212 to 1 litre medium | |

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**Reference:**

Bolton, F.J., Hutchinson, D.N., Parker, G. (1988). Reassessment of Selective Agars and Filtration Techniques for Isolation of Campylobacter Species from Faeces. Eur. J. Clin. Microbiol. Infect. Dis. 7: 155-160.

## *VPT (Skirrow's) Selective Supplement*

**X214**

**VANCOMYCIN, POLYMYXIN, TRIMETHOPRIM, to make Skirrow's medium for the isolation of *Campylobacter* spp.**

Suitable for use with LAB001 Columbia Agar or other blood agar bases, supplemented with lysed horse blood.

| Final Concentration | mg/litre |
|---|---|
| Vancomycin | 10 |
| Polymyxin | 2500 iu/litre |
| Trimethoprim | 5 |
| Add 1 vial of X214 to 1 litre of medium | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, along with other additives, mix well and pour.

**Reference:**

Skirrow, M.B. (1977) British Medical Journal 2 11-9.

## *P-INC Supplement - PNCV*

**X219**

**PENICILLIN, NISIN, CRYSTAL VIOLET, for accelerated shelf life determination of dairy products.**

**For smaller volumes X019 is available (1 vial per 200ml)**

The Pre-incubation test uses a selective mixture to inhibit Gram positive organisms whilst allowing the growth of Gram negative bacteria, the main cause of post-pasteurisation contamination and a major factor in determining the shelf life of the product. The technique is also useful for monitoring plant hygiene.

| Final Concentration | mg/litre |
|---|---|
| Penicillin | 20,000iu/litre |
| Nisin | 40,000iu/litre m |
| Crystal violet | 2.0 |
| Add 1 vial of X219 to 1 litre of Milk Agar LAB019 | |

Rehydrate contents of 1 vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, mix thoroughly and pour plates.

**Method A**

Pre-incubate test material at 21˚C for 24hr. Prepare suitable dilution series, and inoculate Milk Agar plates containing P-INC supplement. Incubate at 21˚C for 24hr, and count all colonies (some may be small, use of a hand lens is recommended). Calculate the CFU/ml and using the tables of Griffith's *et al* the shelf life can be determined.

**Method B**

Rehydrate X219 with 1ml of deionised water only, add 0.1ml to the test material and incubate at 20˚C for 24hr. Prepare suitable dilution series, and inoculate Milk Agar plates. Proceed as for Method A above.

**References:**

Griffiths, M.W., and Phillips, J.D. (1985) J.Appl.Bact. 57, 107.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1980) J. Soc. Dairy Technol. 33, 8.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1981) J. Soc. Dairy Technol. 34, 142.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) J. Soc. Dairy Technol. 37, 22.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) Rapid detection of post-pasteurised contamination. Hannah Research Inst. Bulletin No.10.

Griffiths, M.W., and Phillips ,J.D., and Muir, D.D. (1984) Dairy Ind. Int. 50 (3) 25

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1984) Post-pasteurisation contamination - the major cause of failure of fresh dairy products. Hannah Research Inst.

Griffiths, M.W., and Phillips, J.D., and Muir, D.D. (1986) Aust. J. Dairy Technol. 41, 77-79.

## *CFC Supplement*

**X223**

**MODIFIED C.F.C. – CEPHALOTHIN, FUCIDIN, CETRIMIDE for the selective isolation of *Pseudomonas* spp.**

When added to LAB108 Pseudomonas Agar, to prepare C.F.C. medium this supplement can be used to select pseudomonads from food and environmental samples.

For smaller volumes X108 is available (1 vial per 500ml)

| Final Concentration | mg/litre |
|---|---|
| Cephalothin | 50 |
| Fucidin | 10 |
| Cetrimide | 10 |
| Add 1 vial X223 to 2,000ml medium | |

Rehydrate contents of vial with 5ml of sterile 50% alcohol. Add aseptically to sterilised medium cooled to 47˚C, mix gently and pour.

**Reference:**

Mead, G.C. and Adams, B.W. (1977). Br. Poult. Sci. 18: 661-667

## *GC Growth Supplement*

**X271**

**GROWTH SUPPLEMENT, to improve the isolation of *Neisseria* spp. from selective media.**

For addition to GC agar base LAB067.

| Final Concentration | mg/litre |
|---|---|
| L-cystine | 11 |
| L-cysteine | 259 |
| Thiamine HCl | 0.03 |
| Ferric nitrate | 0.2 |
| Co-Carboxylase | 1 |
| NAD | 1.0 |
| Guanine HCl | 0.3 |
| Adenine | 10 |
| L-glutamine | 100 |
| PABA | 0.13 |
| Vitamin B12 | 0.1 |
| Add 1 vial to 1 litre of medium | |

Rehydrate contents of vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C, along with other additives, mix well and pour.

## *Polymyxin B*

**X274**

**POLYMYXIN for the isolation of *B. cereus* from foods.**

Suitable for the preparation of LAB073 Bacillus cereus Medium (P.R.E.P.). The addition of X073 sterile egg yolk emulsion is also required.

For smaller volumes X074 is available (1 vial per 500ml)

| Final Concentration | |
|---|---|
| Polymyxin B | 8mg/litre = 64,000i.u/litre |
| Add 1 vial X074 to 500ml medium | |
| Add 1 vial X274 to 2L medium | |

Rehydrate contents of vial with 5ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with egg yolk emulsion, mix gently and pour.

**Reference:**

Micro-organisms in Food. Ed. Thatcher, F.S., Clarke, D.F. published by Univ. of Toronto Press.

## *Nalidixic Acid*

**X291**

**NALIDIXIC ACID for the isolation of non-sporing anaerobes from clinical material.**

Suitable for use with LAB090 Fastidious Anaerobe Agar. When used with other blood agar bases, e.g. LAB001 Columbia Agar, further enrichment of the medium with haemin, menadione and sodium pyruvate is beneficial. The addition of Polysorbate 80, which, enhances the growth of anaerobic cocci, to the medium is required for N.A.T. medium. The Polysorbate 80 may be added before sterilisation at a concentration of 0.1%.

| Final Concentration | mg/litre |
|---|---|
| Nalidixic acid | 10 |
| Add 1 vial X291 to 1 litre medium | |

Rehydrate contents of each vial with 5ml sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C together with other additives, mix gently and pour.

**Reference:**

Wren, M.W.D., 1980. J. Clin. Path. 33: 61-65. Multiple Selective Media for the isolation of anaerobic bacteria.

## *VCC Supplement*

**X546**

**V.C.C. Supplement for the selective enrichment of *E. coli* O157:H7 from food and other samples.**

For use with Buffered Peptone Water LAB046

| Final Concentration | mg/litre |
|---|---|
| Vancomycin | 8.0 |
| Cefixime | 0.05 |
| Cefsulodin | 10.0 |
| Add 1 vial of X546 to 2.25 litres of LAB046 | |

Rehydrate the contents of one vial with 20ml of sterile deionised water. Add aseptically to sterilised medium cooled to 47˚C. Mix well and dispense into 225ml aliquots.

## *Half Fraser Supplement*

**X564**

**HALF FRASER supplement for the primary enrichment of *Listeria* spp from food and environmental samples.**

For addition to LAB164 Fraser Broth Base

For smaller volumes X164 is available (1 vial per 450ml)

| Final Concentration | mg/litre |
|---|---|
| Ferric ammonium citrate | 500 |
| Acriflavine | 12.5 |
| Nalidixic acid | 10 |
| Add 1 vial of X564 to 2.25 litres of Fraser Broth Base | |

Rehydrate contents of vial with 2ml 50% methanol (5ml for X564). Add aseptically to sterilised medium cooled to 47˚C, mix well and pour.

# 9. OrganoTone™ Media Constituents

**Sourcing**

The Lab M range of media constituents are selected on the basis of quality and performance from the world's leading suppliers. It is a deliberate policy not to invest in our own peptone manufacturing facility in order to allow our microbiologist freedom to choose the best ingredients available on the international market.

**Agars**

A range of agars are offered to suit all microbiological applications. Koch originally used gelatin to solidify culture media, but the superior properties of agar resulted in its universal adoption as the gelling agent of choice. Careful selection of agars is vital as they can interact with nutrient components in a beneficial or deleterious manner.

**Peptones and Extracts**

Like agars, peptones and extracts are biologically variable products requiring careful selection. They provide the amino acids and peptides required by micro-organisms for growth as well as other vital growth factors such as minerals, vitamins and nucleic acid fractions.

To ensure we use only the best available peptones and extracts these materials are exhaustively tested. Growth parameters are obtained by classical microbiological techniques and by automated growth rate analysis. Chemical and physical properties are also closely monitored. Lab M can select specific peptones for special purposes such as vaccine production and fermentation processes. If more information is required on special services please contact Lab M or your local agent.

## Acid Hydrolysed Casein

**MC007**

A soluble protein hyrolysate obtained by digesting casein with hot acid. It is almost free from growth factors, vitamins and antagonists, and these qualities make it suitable for use as a protein source in media for antibiotic and vitamin assays.

**Typical Analysis**

| | |
|---|---|
| Appearance | cream/white powder |
| Solubility in water at 5% | total |
| Clarity | clear and colourless |
| pH of 2% solution | 6.0 ± 0.5 |
| Total Nitrogen | 8.3% ± 0.5 |
| Total Amino Nitrogen | 6.1% ± 0.5 |

## Agar No. 1 Bacteriological

**MC002**

A high clarity agar with good gelling properties and a low concentration of metal ions. This agar is suitable for all bacteriological purposes including sensitivity testing and pour plate techniques. A firm gel is obtained at working concentrations of 1.0 to 1.5%. No significant precipitation is observed on reheating or prolonged holding at 65˚C.

**Typical Analysis**

| | |
|---|---|
| Gel strength (Nikan) | 650-1000g/m2 |
| Colourimetry (1.5% soln at 65˚C) | > 0.28 at 340nm |
| | > 0.02 at 525nm |
| Melting point | > 85˚C |
| Setting point | 32-35˚C |
| pH | 6.5-7.4 |
| Moisture | < 10% |
| Total ash | < 3% |
| Calcium | < 0.02% |
| Magnesium | < 0.02% |
| Sodium chloride | < 1.0% |
| Iron | < 0.01% |
| Insoluble ash | < 0.1% |
| Sulphate | 1.5% |
| *Salmonella* | Absent |
| TVC | $< 10^3$/g |
| Spores | < 2/g |

## Agar No. 2 Bacteriological

**MC006**

A bacteriological agar which gives a firm gel at working concentrations of 1.0 to 1.5% which is reasonably clear. This agar is recommended for all culture media except sensitivity testing media and those where absolute clarity is advantageous.

**Typical Analysis**

| | |
|---|---|
| Gel strength (Nikan) | 650-1000g/$m^2$ |
| Colourimetry (1.5% soln at 65˚C) | > 0.3 at 340nm |
| | >0.04 at 525nm |
| Appearance | cream/white powder |
| Melting point | >85˚C |
| Setting point | 32-35˚C |
| pH | 6.5-7.4 |
| Moisture | <12% |
| Total ash | <3.5% |
| Calcium | < 0.5% |
| Magnesium | < 0.1% |
| Sodium chloride | <1.0% |
| Iron | < 0.01% |
| Insoluble ash | < 0.1% |
| Sulphate | < 3.0% |
| *Salmonella* | Absent |
| TVC | $< 10^3$/g |
| Spores | < 2/g |

## *Agar No.4*

***Plant Tissue Culture Grade***

**MC029**

Lab M Agar No.4 has been selected specifically for use as a gelling agent in plant tissue culture techniques. The product is selected primarily on gel strength, a parameter of particular importance for this application, and then tested to ensure it meets the parameters set by a major plant producer. The agar contains no nutrients for plant growth and is designed to be incorporated into classical formulations such as Murashige and Skoog, as well as customer's own formulations.

| **Typical analysis** | |
|---|---|
| Ash | 2.30% |
| Acid Insoluble Ash | 0.16% |
| Calcium | 0.31% |
| Magnesium | 0.12% |
| Iron | 0.018% |
| Total Nitrogen | 0.15% |
| Recommended Concentration | 0.75 - 1.5% |
| Melting Point | 88-91˚C |
| Setting Point | 32-33˚C |
| Mesh | 80 |
| pH (1.5% at 20˚C) | 7.0 ± 0.2 |
| Gel Strength (1.5% W/V) | >700g/cm$^2$ |

## *Bacteriological Peptone*

**MC024**

An economical source of nutrients provided by a balanced mixture of meat peptones and tryptone. The growth requirements of most non fastidious organisms will be fulfilled by the range of amino acids, peptides and proteoses in this mixture.

| **Typical Analysis** | |
|---|---|
| Appearance | cream powder |
| Solubility in water at 5% | total |
| Clarity | clear, pale straw colour |
| pH of 2% solution | 7.2 ± 0.2 |
| Total Nitrogen | 12% ± 0.5 |
| Amino Nitrogen | 5% ± 0.5 |

## *Balanced Peptone No. 1*

**MC004**

A rich mixture of tryptone and meat peptones which fulfills the nutritional demands of a wide variety of micro-organisms. This peptone is used in many Lab M culture media formulations.

| **Typical Analysis** | |
|---|---|
| Appearance | beige powder |
| Solubility in water at 5% | total |
| Clarity | clear, pale straw colour |
| pH of 2% solution | 7.2 ± 0.2 |
| Total Nitrogen | 12.8% ± 0.5 |
| Amino Nitrogen | 5.1% ± 0.5 |

## *Beef Extract*

**MC019**

This complements the nutritive properties of peptones in culture media and is often used as an added enrichment. Beef extract can be used as a direct replacement for meat peptones and, as it contains no carbohydrates, can be used as a component of media for fermentation studies.

| **Typical Analysis** | |
|---|---|
| Appearance | light brown powder |
| Solubility in water at 5% | total |
| Clarity | clear, light brown colour |
| pH of 2% solution | 7.0 ± 0.2 |
| Total Nitrogen | 12.0% ± 0.5 |
| Amino Nitrogen | 1.6% ± 0.5 |

## *Bile Salts No. 3*

**MC025**

A refined bile salt, comprising mainly sodium cholate and sodium desoxycholate. It is used as a selective agent in culture media such as Violet Red Bile Agar for the enumeration of coliforms, MacConkey Agar No. 3 for the isolation and differentiation of enteric organisms, and SS Agar for the isolation of enteric pathogens. This fraction of bile is highly active, allowing maximum selection of organisms of enteric origin at relatively low concentrations (0.15%).

| **Typical Analysis** | |
|---|---|
| Appearance | white powder |
| Solubility in water at 2% | total |
| Clarity | clear |
| pH of a 2% solution | 8.0 ± 0.5 |

## *FMV (Foot and Mouth Vaccine) Peptones*

**MC033**

**Description**

A special blend of peptones developed for use in the production of Foot and Mouth vaccine.

| **Typical analysis:** | |
|---|---|
| Appearance | cream powder |
| Solubility in water at 2% | total |
| Clarity | clear, straw colour |
| pH of 2% solution | 7.0 ± 0.2 Total |
| Nitrogen | 10.0 - 14.5% |
| Amino Nitrogen | 2.5 - 5.5% |

## *Gelatin Powder*

**MC015**

**Description**
A collagenous protein used for the solidification of culture media and for the detection and differentiation of certain proteolytic bacteria.

| **Typical Analysis:** | |
|---|---|
| Appearance | buff crystalline powder |
| pH | 5.7 |

## *Glucose (Dextrose)*

**MC013**

**Description**
Glucose for use in microbiological culture media.

## *IPTG (Isopropyl-β-D-Thiogalactopyranoside)*

**MC402**

**Description**
Isopropyl-β-D-Thiogalactopyranoside (IPTG) is used as an inducer of the lac Z operon. Incorporation of this compound into media containing X-β-Galactoside (MC405), or equivalent chromogenic compound, enhances the colour development of organisms capable of fermenting lactose. This combination of compounds is especially useful in transformation experiments involving *Escherichia coli* where disruption of the lac Z operon is used as a marker for DNA insertion.

## *Lactalbumin Hydrolysate*

**MC040**

**Description**
Lactalbumin is a protein removed from the whey, left after removal of casein from milk. Lactalbumin hydrolysate is a pancreatic digest of these proteins, containing high levels of essential amino acids. It can be used for tissue culture media and for production of vaccines of viral origin. Other uses include growth of lactobacilli, clostridial spores and certain fermentation procedures.

| **Typical analysis:** | |
|---|---|
| Appearance | cream powder |
| Solubility in water at 5% | total |
| Clarity | clear, pale straw colour |
| pH of 2% solution | 7.0 ± 0.2 |
| Total Nitrogen | 12.0% ± 0.5 |
| Amino Nitrogen | 5.5 ± 0.5 |

## *Lactose*

**MC020**

**Description**
Lactose for use in microbiological culture media.

## *Lecithin (Soya Bean Lecithin)*

**MC041**

**Description**
A deoiled, powdered soybean lecithin for use in microbiological culture media.

**Composition**
A mixture of polar (phospho- and glyco-) lipids and a small amount of carbohydrates.

| | | |
|---|---|---|
| Acetone insolubles | min. | 97% |
| Oil | max. | 1.5% |
| Phosphatidylcholine | | 20-24% |
| Phosphatidylethanolamine | | 18-22% |
| Phosphatidylinositol | | 12-15% |
| Phosphatidic acid | | 4-7% |
| Fatty acids (total content) including saturated fatty acids, monounsaturated fatty acids & polyunsaturated fatty acids | approx. | 50% |

| **Typical analysis:** | |
|---|---|
| pH (1% aqueous solution) | 6-7 |
| Total plate count | <1000/g |
| Solubility in: | |
| water | dispersible |
| fats / oils | soluble |

**Appearance**
Fine, beige powder

**Hazard classification**
NR – Not regulated

**Storage**
Store at 10-25˚C. Do not store above 25˚C.

This component is suitable for use in the preparation of microbiological culture media. Other uses and applications are subject to validation by the end user.

## *Liver Digest*

**MC034**

Liver digest is prepared by the controlled hydrolysis of liver. It is rich in vitamins and essential amino acids and has excellent nutritional properties and especially favours the growth of strict anaerobes and other fastidious microorganisms. Due to its capacity to stimulate sugar metabolism in saccharolytic organisms it is perfectly suited for the growth of a broad range of organisms such as *Clostridium*, *Leuconostoc*, *Bacillus*, homo and hetero-fermentative Lactic acid bacteria, as well as yeasts and filamentous moulds.

| **Typical Analysis** | |
|---|---|
| Appearance | light brown powder |
| Solubility in water at 5% | total |
| Clarity | clear, brown colour |
| pH of 2% solution | 6.0 - 7.0 |
| Total Nitrogen | 9.5 - 11.5% |
| Amino Nitrogen | 4.0 - 6.0% |

**Storage**
Dehydrated culture media: 10-25°C away from direct sunlight.

## *Malt Extract*

**MC023**

A water soluble extract of malted barley suitable for use in the cultivation of yeasts and moulds. Malt extract has a very high carbohydrate content and consequently is very sensitive to over heating which will cause a darkening of the medium.

**Typical Analysis**

| | |
|---|---|
| Appearance | yellow/brown powder |
| Solubility in water at 5% | total |
| Clarity | clear, light brown colour |
| pH of 2% solution | 5.2 ± 0.2 |
| Maltose | 55% |
| Other Carbohydrates | 40% |
| Protein | 5% |

## *Maltose Monohydrate*

**MC022**

**Description**
Maltose for use in microbiological culture media.

## *Mannitol*

**MC014**

**Description**
D-Mannitol for use in microbiological culture media.

## *Meat Peptone*

**MC018**

**Description**
A highly nutritious enzymatic digest of meat for use in microbiological culture media

**Typical Analysis:**

| | |
|---|---|
| Appearance | cream powder |
| Solubility in water at 5% | total |
| Clarity | clear, pale straw colour |
| pH of 2% solution | 6.7 - 7.3 |
| Total Nitrogen | 11.0 - 13.0% |
| Amino Nitrogen | 2.5 - 5.0% |

## *MUG (4-Methylumbelliferyl-ß-D-Glucuronide)*

**MC406**

**Description**
MUG is a fluorogenic compound used for the specific detection of *E. coli* in bacteriological culture media. MUG reagent is cleaved by the enzyme glucuronidase to release fluorescent 4-methylumbelliferone that can be detected under long wave UV light (366nm) as a blue/green fluorescence. MUG can be incorporated into a range of culture media to enhance detection of *E. coli*.

## *Mycological Peptone*

**MC009**

A mixture of peptones with a high carbohydrate content suitable for the rapid growth and colonial development of yeasts and moulds. Bacterial growth is inhibited by the low pH of this peptone.

**Typical Analysis**

| | |
|---|---|
| Appearance | beige powder |
| Solubility in water at 5% | total |
| Clarity | clear, pale straw colour |
| pH of 2% solution | 5.4 ± 0.1 |
| Total Nitrogen | 13% ± 0.5 |
| Amino Nitrogen | 1.4% ± 0.5 |

## *Ox Bile*

**MC010**

**Description**
Ox bile is a dehydrated, purified fresh bile used as a selective agent in bile media, such as Brilliant Green Bile 2% Broth (LAB051).

## *Proteose Peptone A*

**MC011**

An enzymatic digest of meat adapted to encourage the production of toxins by *Corynebacterium diphtheriae,* staphylococci, salmonellae, and clostridia. This peptone is highly nutritious and suitable for use in culture media for fastidious organisms such as *Neisseria, Haemophilus* and *Pasteurella* species.

**Typical Analysis**

| | |
|---|---|
| Appearance | cream powder |
| Solubility in water at 5% | total |
| Clarity | clear, light straw colour |
| pH of 2% solution | 7.0 ± 0.2 |
| Total Nitrogen | 12.0% ± 0.5 |
| Amino Nitrogen | 5.8% ± 0.5 |

## *Skim Milk Powder*

**MC027**

A bacteriological grade of thermophile free spray dried skim milk. Used in Milk Plate Count Agar LAB115 and in media for diagnostic tests involving the digestion or coagulation of casein and the fermentation of lactose. Recommended working concentration 10%.

| Typical Analysis | |
|---|---|
| Appearance | white powder |
| Clarity | opaque white suspension |
| Total Nitrogen | 5.3% ± 0.5 |
| Lactose | 48.0% ± 0.5 |

## *Sodium Chloride (Bacteriological)*

**MC017**

**Description**
Sodium chloride for use in microbiological culture media.

## *Sodium Desoxycholate*

**MC026**

Sodium desoxycholate is a specific bile acid, derived from deconjugated bile salts. Leifson showed that desoxycholic acid had the most inhibitory effect on bacterial growth, and that this could be enhanced by the removal of magnesium ions by chelating with sodium citrate. These components comprise the selective agents in DCA, DCA (Hynes) and DCLS.

| Typical Analysis | |
|---|---|
| Appearance | white powder |
| pH of 2% solution | 8.3 ± 0.5 |
| Solubility in water at 2% | total |
| Moisture | <5% |
| Heavy metals | <20 ppm |
| Sodium cholate | <2% |

## *Sodium Thioglycollate*

**MC016**

**Description**
Sodium thioglycollate for use in bacteriological culture media. It is used to lower the oxidation-reduction potential of the medium and for the neutralisation of mercurial compound preservatives.

## *Soy Peptone*

**MC003**

Prepared using the enzyme papain to digest soyabean meal, this peptone is a rich source of nutrients with a high carbohydrate content. Most organisms will grow rapidly in this peptone but some bacteria will produce high levels of acid leading to auto-sterilisation unless an adequate buffering system is incorporated.

| Typical Analysis | |
|---|---|
| Appearance | cream powder |
| Solubility in water at 5% | total |
| Clarity | clear, straw colour |
| pH of 2% solution | 7.1 ± 0.2 |
| Total Nitrogen | 9.0% ± 0.5 |
| Amino Nitrogen | 1.6% ± 0.5 |

## *Tryptone*

**MC005**

An enzymatic hydrolysate of casein, rich in peptones and amino acids (including tryptophane). This peptone can be utilised by most bacteria as a growth substrate.

| Typical Analysis | |
|---|---|
| Appearance | cream powder |
| Solubility in water at 5% | total |
| Clarity | clear, pale straw colour |
| pH of 2% solution | 7.2 ± 0.5 |
| Total Nitrogen | 13.0% ± 0.5 |
| Amino Nitrogen | 4.9% ± 0.5 |

## *Tryptose*

**MC008**

A blend of peptones suitable for the cultivation of most fastidious organisms including *Neisseria gonorrhoeae, Streptococcus milleri* and *Brucella* spp. especially where rapid or profuse growth is required such as in blood culture media and blood agars.

| Typical Analysis | |
|---|---|
| Appearance | beige powder |
| Solubility in water at 5% | total |
| Clarity | clear, light straw colour |
| pH of 2% solution | 7.2 ± 0.2 |
| Total Nitrogen | 12.5% ± 0.5 |
| Amino Nitrogen | 4.9% ± 0.5 |

## *X-β-Galactoside (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside)*

**MC405**

**Description**

X-β-galactoside (5-Bromo-4-chloro-3-indolyl-β-D-galactoside) is used as a chromogenic substrate for the detection of organisms capable of fermenting lactose (lac Z-positive organisms). In combination with IPTG (MC402), X-β-Galactoside can be used in transformation experiments involving *Escherichia coli* where disruption of the lac Z operon is used as a marker for DNA insertion.

## *Yeast Extract Powder*

**MC001**

Prepared by the autolysis of *Saccharomyces cerevisae* under thermostatically controlled conditions to protect the B vitamins and other heat labile constituents. This extract provides a mixture of amino acids, peptides, vitamins and carbohydrates making it suitable for many applications.

| **Typical Analysis** | |
|---|---|
| Appearance | yellow powder |
| Solubility in water at 5% | total |
| Clarity | clear, pale yellow |
| pH of 2% solution | 7.0 ± 0.2 |
| Total Nitrogen | 10.5% ± 0.5 |
| Amino Nitrogen | 5.3% ± 0.5 |

| TYPICAL ANALYSIS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **MC007 Acid Hydrolysed Casein** | **MC024 Bacteriological Peptone** | **MC004 Balanced Peptone No. 1** | **MC009 Mycological Peptone** | **MC011 Proteose Peptone A** | **MC003 Soy Peptone** | **MC005 Tryptone** | **MC008 Tryptose** | **MC001 Yeast Extract** |
| Total Nitrogen | 8.3% | 12.0% | 12.5% | 12.6% | 12.0% | 9.0-9.4% | 12.8% | 12.7% | 10.5% |
| Total AminoNitrogen | 6.1% | 5.0% | 5.1% | 1.4% | 5.8% | 1.6-1.8% | 4.8% | 4.9% | 5.3% |
| Amino N/Total N. | 73.4% | 41.7% | 40.8% | 11.1% | 48.0% | 17.7% | 37.5% | 38.7% | 50.4% |
| **Total Amino Acid Assay (mg/g)** | | | | | | | | | |
| Lysine | 53.5 | 62.0 | 75.3 | 38.9 | 48.2 | 43.1 | 79.7 | 77.5 | 49.0 |
| Histidine | 18.7 | 21.0 | 26.8 | 12.7 | 15.6 | 16.2 | 31.2 | 29.0 | 14.0 |
| Arginine | 22.1 | 32.0 | 41.0 | 55.0 | 50.6 | 39.2 | 37.5 | 39.2 | 27.0 |
| Aspartic Acid | 44.1 | 69.0 | 58.4 | 69.9 | 63.9 | 74.4 | 62.8 | 60.6 | 52.0 |
| Threonine | 23.5 | 40.0 | 31.1 | 17.7 | 27.5 | 21.7 | 36.9 | 34.0 | 33.0 |
| Serine | 30.0 | 40.0 | 40.3 | 26.2 | 36.2 | 27.0 | 50.3 | 45.3 | 34.0 |
| Glutamic Acid | 130.0 | 160.0 | 138.9 | 103.5 | 100.2 | 110.0 | 184.0 | 161.5 | 73.0 |
| Proline | 52.2 | 46.0 | 61.0 | 74.5 | 55.6 | 28.0 | 82.1 | 71.6 | 26.0 |
| Glycine | 11.1 | 29.0 | 44.5 | 105.9 | 83.38 | 22.6 | 15.6 | 30.0 | 25.0 |
| Alanine | 19.0 | 39.0 | 38.1 | 49.9 | 52.3 | 23.1 | 26.9 | 32.5 | 51.0 |
| Cystine | 1.1 | 1.0 | 1.1 | 2.7 | 8.4 | 5.3 | 2.2 | 1.6 | 6.0 |
| Valine | 35.2 | 45.0 | 45.3 | 22.9 | 35.2 | 23.7 | 59.2 | 52.3 | 37.0 |
| Methionine | 10.4 | 8.0 | 19.1 | 7.0 | 12.3 | 6.2 | 25.0 | 22.1 | 9.0 |
| Isoleucine | 27.9 | 33.0 | 43.8 | 18.7 | 23.3 | 26.8 | 58.5 | 51.1 | 73.0 |
| Leucine | 30.9 | 65.0 | 65.3 | 32.0 | 55.5 | 38.6 | 83.5 | 74.4 | 73.0 |
| Tyrosine | 12.7 | 12.0 | 9.8 | 12.8 | 13.3 | 16.8 | 14.7 | 12.2 | 12.0 |
| Phenylalanine | 17.0 | 34.0 | 31.8 | 20.2 | 27.2 | 22.7 | 42.4 | 37.1 | 25.0 |
| Tryptophane | - | 3.0 | 4.9 | 1.9 | 4.6 | 3.7 | 6.6 | 5.7 | 9.0 |

# 10. Sterile Additives

Sterile additive products are offered in ready-to-use format. Each product has been prepared with an appropriate sterilisation procedure, e.g. aseptic preparation, filtration or irradiation, which will not affect the performance of the product. All sterile reagents should be stored at 2-8ºC away from light.

The ready prepared media offer a cost effective alternative to preparation of large quantities of small volume dispensed media.

## HAL010 Listeria Selective Diagnostic Supplement

**X010**

**Description**

A selective diagnostic supplement containing cycloheximide, nalidixic acid and phosphatidylinostiol for the isolation and presumptive identification of *Listeria monocytogenes*. For use with HAL010 Harelquin™ Listeria Chromogenic Agar.

For larger volumes X210 is available (2.5 litres per vial)

**Final Concentration**

| | Concentration Per vial X010 | Conc. in medium (mg/litre) |
|---|---|---|
| Cycloheximide | 25 mg | 50 |
| Nalidixic acid | 10 mg | 20 |
| Phosphatidylinositol | ~300 mg | ~600 |

**Instructions for use**

Pre-heat X010 to 48-50°C and aseptically add to sterilised medium cooled to 48-50°C.

Add 1 vial X010 and 1 vial of X072 to 475mL medium

**References**

ISO 11290-1:1997 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Listeria monocytogenes* - Part 1: Detection method. Incorporating Amendment 1.

## Potassium Tellurite Solution 3.5%

**X027**

**Description**

A sterile solution of 3.5% potassium tellurite. A selective agent for addition to Hoyles's Medium (Modified) (LAB027), for the selective isolation and differentiation of *Corynebacterium diphtheriae*.

**Directions**

Add 1 vial (2ml) to 200ml of Hoyle's Medium (LAB027).

## Potassium Lactate

**X034**

**Description**

A sterile solution of 10% potassium lactate for use with LAB201 Lysine Medium, for the isolation and enumeration of wild yeasts in pitching yeasts.
For larger volumes X035 is available (1 vial per 500ml)

**Directions**

Add 1 vial X034 (1ml) Potassium Lactate to 100ml (10ml/litre) hydrated LAB201 Lysine Medium prior to sterilisation.

## Potassium Lactate

**X035**

**Description**

A sterile solution of 10% potassium lactate for use with LAB201 Lysine Medium, for the isolation and enumeration of wild yeasts in pitching yeasts.
For smaller volumes X034 is available (1 vial per 100ml)

**Directions**

Add 1 vial X035 (5ml) Potassium Lactate to 500ml (10ml/litre) hydrated LAB201 Lysine Medium prior to sterilisation.

## Lactic Acid 10%

**X036**

**Description**

A sterile solution of 10% lactic acid added to culture media to reduce the pH, in order to suppress bacterial growth.

For larger volumes X037 is available (5ml)

**Directions**

Add 5 vials (5ml) to 1 litre of Malt Extract Agar LAB037.

Add 10 vials (10ml) to 1 litre of Potato Dextrose Agar LAB098.

Add 1 vial (1ml) to 1 litre of Lysine Agar LAB201.

Addition of X037 should be carried out after sterilisation and cooling the medium to 47ºC.

## Lactic Acid 10%

**X037**

**Description**

A sterile solution of 10% lactic acid added to culture media to reduce the pH, in order to suppress bacterial growth.

For smaller volumes X036 is available (1ml)

**Directions**

Add 1 vial (5ml) to 1 litre of Malt Extract Agar LAB037.

Add 2 vials (10ml) to 1 litre of Potato Dextrose Agar LAB098.

Addition of X037 should be carried out after sterilisation and cooling the medium to 47ºC.

## Potassium Tellurite Solution 1%

**X043**

**Description**

Potassium Tellurite Solution for use with Modified Giolitti and Cantoni Broth (ISO) LAB219 for the detection and enumeration of coagulase-positive staphylococci.

| Formulation | Per vial | Conc. in medium |
|---|---|---|
| Potassium tellurite | 10 g/L | 0.1 g/L |

**Addition**

Aseptically add to media tempered to 44-47ºC sufficient volume of X043 1% potassium tellurite to give a final concentration of 0.1g/L. For example, add 0.1ml X043 to 9ml of single strength base or add 0.2ml X043 to 10ml of double strength base.

**Appearance**

Clear, colourless liquid

**References**

ISO 6888-3:2003 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 3: Detection & MPN technique for low numbers.

## *Egg Yolk Emulsion*

**X073**

**For larger volumes X573 is available (500ml)**

**Description**

A sterile emulsion of egg yolks for use in bacteriological culture media. It may be added directly to nutrient media for the identification of *Clostridium, Bacillus* and *Staphylococcus* species by their lipase and/or lecithinase activity.

Presented in 100ml bottles, add 100ml to 900ml of Bacillus cereus medium (PREP and PEMBA, LAB073 and LAB193), 40 ml to Brazier's CCEY Agar LAB160, or 50ml to Blood Agar Base LAB028 containing Fildes extract and serum.

**Technique**

For detection of lecithinase activity (especially in the investigation of `bitty cream' conditions) add 0.5 or 1.0ml of the emulsion to 10ml of sterile Blood Agar Base (LAB028) or Nutrient Broth No.2 (LAB014). In order to clear the medium, raise the final salt concentration by the addition of 1% of sodium chloride. After incubation for up to 5 days at 35ºC, lecithinase-producers render the broth opalescent, whilst, on the solid medium, the colonies are surrounded by opaque zones.

## *Egg Yolk Tellurite Emulsion*

**X085**

**Description**

A sterile egg yolk emulsion containing potassium tellurite for use in Baird-Parker Medium Base (ISO) LAB285 or Baird-Parker Medium Base LAB085.

| Formulation | mg/litre |
|---|---|
| Potassium tellurite | 0.20% w/v |
| Egg yolk emulsion | 20% |
| Final concentration of tellurite in medium is 0.01% w/v. | |

**Appearance**

Yellow, opaque solution with resuspendable deposit.

**Storage**

Once opened store at 2-8°C. A deposit may form during storage. This is normal and will not affect the product. Mix well before use.

**Method for reconstitution**

Add 50mL (5%) X085 to 1 litre of sterilised media, tempered to 48ºC.

## *Urea Solution 40%*

**X130**

**Description**

A sterile solution of 40% urea, for addition to Urea Broth Base (LAB131) and Urea Agar Base (LAB130) for the detection of urease production by *Proteus* spp.

For larger volumes X135 is available (100ml)

**Directions**

Add 1 vial X130 (5ml) to 95ml of Urea Broth Base (LAB131) and Urea Agar Base (LAB130).

## *Urea Solution 40%*

**X135**

**Description**

A sterile solution of 40% urea, for addition to Urea Broth Base (LAB131) and Urea Agar Base (LAB130) for the detection of urease production by *Proteus* spp.

For smaller volumes X130 is available (5ml)

**Directions**

Add 5ml to 95ml of Urea Broth Base (LAB131) and Urea Agar Base (LAB130).

## *HAL010 Listeria Selective Diagnostic Supplement*

**X210**

**Description**

A selective diagnostic supplement containing cycloheximide, nalidixic acid and phosphatidylinostiol for the isolation and presumptive identification of *Listeria monocytogenes*. For use with HAL010 Harelquin™ Listeria Chromogenic Agar.

For smaller volumes X010 is available (500 mL per vial)

| Final Concentration | Concentration Per vial X210 | Conc. in medium (mg/litre) |
|---|---|---|
| Cycloheximide | 125 mg | 50 |
| Nalidixic acid | 50 mg | 20 |
| Phosphatidylinositol | ~1500 mg | ~600 |

**Instructions for use**

Pre-heat X210 to 48-50°C and aseptically add to sterilised medium cooled to 48-50°C.

Add 1 vial of X210 and 5 vials of X072 to 2375mL medium.

**References**

ISO 11290-1:1997 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Listeria monocytogenes* - Part 1: Detection method. Incorporating Amendment 1.

## *Egg Yolk Emulsion*

**X573**

**For smaller volumes X073 is available (100ml)**

**Description**

A sterile emulsion of egg yolks for use in bacteriological culture media. It may be added directly to nutrient media for the identification of *Clostridium, Bacillus* and *Staphylococcus* species by their lipase and/or lecithinase activity.

Presented in 100ml bottles, add 100ml to 900ml of Bacillus cereus medium (PREP and PEMBA, LAB073 and LAB193), 40 ml to Brazier's CCEY Agar LAB160, or 50ml to Blood Agar Base LAB028 containing Fildes extract and serum.

**Technique**

For detection of lecithinase activity (especially in the investigation of `bitty cream' conditions) add 0.5 or 1.0ml of the emulsion to 10ml of sterile Blood Agar Base (LAB028) or Nutrient Broth No.2 (LAB014). In order to clear the medium, raise the final salt concentration by the addition of 1% of sodium chloride. After incubation for up to 5 days at 35ºC, lecithinase-producers render the broth opalescent, whilst, on the solid medium, the colonies are surrounded by opaque zones.

# *NOTES*

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## Harmonised Pharmacopoeia

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# NEWS

## LAB M LAUNCHES PINNACLE™ GVPC AND BCYE FOR LEGIONELLA TESTING

We are proud to expand our Pinnacle™ range with the addition of pre-poured plates for Legionella GVPC and BCYE. Both are ISO formulation compliant and performance compliant according to BS EN ISO 11133:2014 to support laboratories to meet their QC requirements, including the revised panel of non-target organisms and the complete inhibition of *Enterococcus faecalis*. This supports laboratories for their first UKAS accreditation following the implementation of the new BS EN ISO 11133:2014.

## FOLLOWING THE REVISED ISO 11133:2014

Microbiology laboratories testing food, animal feed or water samples using ISO formulations are now required to follow the latest BS EN ISO 11133:2014 standard which applies to QC testing criteria. This impacts any labs preparing their media in-house, but also to media manufacturers such as ourselves. Not only are Lab M compliant to the new standard, but our SOP's are written in a way to ensure we go above and beyond these criteria to ensure our customers have peace-of-mind when purchasing our products.

## LAB M GAINS ISO 17025 ACCREDITATION FROM UKAS

We're pleased to announce that the quality control laboratory of our facilities in Heywood, has been granted BS EN ISO 17025:2005 accreditation by UKAS. Lab M's schedule of accreditation covers both the physical and microbiological performance testing of our ready-to-use Pinnacle™ media range, with our accredited methods including pH, sterility, fill volume, qualitative performance testing and quantitative performance testing.
All methods are based on the new requirements of BS EN ISO 11133:2014.

## LAB M'S HARMONISED PHARMACOPOEIA RANGE IS NOW AVAILABLE

We have enhanced our pharmacopoeia range to ensure that all media listed in the European pharmacopoeia 8.0 volume 1 (2014) are available in our portfolio. This range of dehydrated culture media is formulated to and performance tested by the requirements specified within the European pharmacopoeia, enabling laboratories to comply to the standard which is harmonised with the equivalent chapters of the United States (USP) and Japanese pharmacopoeias (JP).

## LAB M JOINS NEOGEN

August 2015 saw the acquisition of Lab M by worldwide food and animal safety specialists, Neogen Corporation (NASDAQ: NEOG). Lab M's existing range of microbial testing and diagnostic products complement Neogen's food safety capability perfectly. The future looks bright for both companies as they drive forward their R&D and new product development efforts to provide new and innovative solutions for food safety, water, industrial and clinical markets.

Lab M's current facility will be maintained, and its operations will be managed by Neogen's Scotland-based Neogen Europe subsidiary. Neogen Europe services the Europe, Middle East and Africa (EMEA) territories and has over 160 employees.


LAB™ A Neogen® Company

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